Review Article

IMMUNOTHERAPY FOR CANCER WITH SPECIAL REFERENCE TO LUNG CANCER

Sundeep Kumar Upadhyaya

Consultant, Department of Rheumatology, Indraprastha Apollo Hospitals, Sarita Vihar, New Delhi 110 076, India.

e-mail: sundeepupadhyaya@hotmail.com

Immunotherapy with recombinant antibodies and vaccines are finding uses in a variety of disorders, notably those of Rheumatoid arthritis, Systemic lupus erythematosus and Multiple sclerosis. Malignancies are the latest beneficiaries of this unique form of medicine and there are few a trials (DC vaccines is one of them) that are being conducted at Indraraprastha Apollo Hospitals for treatment of cancer.

Key words: Immunotherapy, Malignancy, Lung cancer.

INTRODUCTION

LUNG cancer is the most important tumor world-wide- it is the leading cause of cancer-related deaths in men and women of most of the developed countries. Indeed in many developing world countries like India, lung cancer morbidity & mortality are on the rise [1]. In fact it accounts for 28% of all cancer fatalities in the US [2]. Under most circumstances, lung cancer is not curable. Current treatment modalities include surgery, chemotherapy and radiation in an attempt to destroy cancer cells or prevent further tumor growth. But these are palliative in most patients because the tumor is usually far advanced. There has therefore been a resurgence of interest in the immunological aspects of lung cancer and how this knowledge might be used for a better outcome [3].

In the middle of the last century, experiments that evaluated the role of immunity against cancer were carried out on inbred mice. Some of the initial studies [4] were based on advances made in transplantation biology. Firstly, methylcholanthrine-induced sarcomas were developed in strains of inbred mice and various cell lines of these sarcomas were established. It was found that when these sarcomas were introduced into other syngeneic mice (mice genetically identical to the ones in which sarcomas were induced), these tumors would grow (*Fig*. 1). In the next step these tumors were resected surgically after some days (step 1). When the same sarcoma tumor cell line was re-introduced into the mice which had had the tumor recently resected surgically (step 2), there was tumor rejection, perhaps revealing the presence of adaptive tumor immunity to the sarcoma. When another sarcoma tumor cell line (distinct from the cell line that induced tumor immunity in the first instance) was introduced into the same syngeneic mice (step 3), the mice were not able to reject the tumor and the tumor grew. These sets of experiments prove that adaptive tumor immunity is at work in the rejection of tumors in a simple yet logical way [4]. To understand more about the tumor specific immune responses and the nature of tumor antigens in lung cancer, more recently, attempts have been made to establish lung cancer cell lines & propagate them in culture [5].

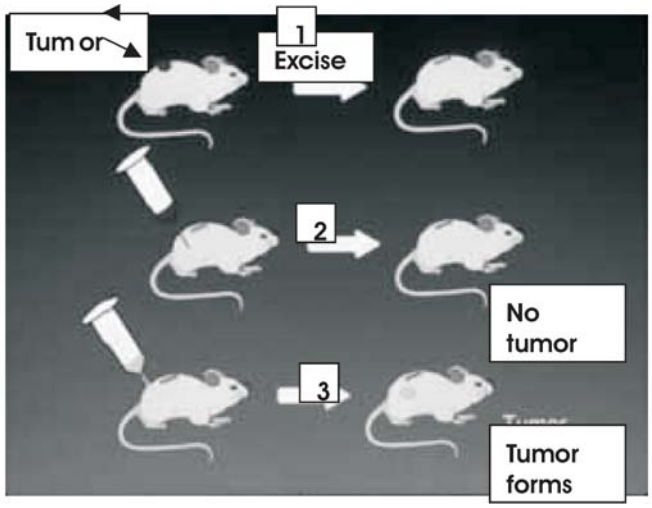

Fig. 1. Historical perspective of immunological response to cancer.

There is also interest in the immunological role of cytokines in studies of tumor immunity. One group of researchers has demonstrated the complete tumor regression of pulmonary metastasis of a melanoma cell line B16 BL6-D5 ("D5") and demonstrated the importance of soluble factors of immunity in tumor rejection. They used cytotoxic T cells that were double knockouts for both Perforin and Gamma Interferon and applied these to wild type animals that had pulmonary metastatic melanoma. Such double knockout T Cells were able to control tumor [6] showing that the expression of cytokines on the wild type animals was enough for tumor rejection. Interestingly though, a similar transfer of these double knockout cytotoxic T cells, was unsuccessful in it's ability to ablate tumor in both Perforin (PKO) and Interferon Gamma knockout (GKO) animals. This establishes the importance of Perforin, Interferon-gamma and Tumor Necrosis Factor-alpha (TNF-alpha) - all mediators of adaptive cellular immunity- as a critical triad of effector molecules involved in host response to tumor [6].

There are also intensive studies on the methods of tumor evasion that is, how tumors adapt to the immune attack and escape rejection (*Fig*. 2). Neoplastic cells are able to escape the host's immune responses by inducing apoptosis in the Cytotoxic T-lymphocytes (CTLs). This is triggered by the interaction of the membrane receptor Fas (CD-40) with its normal ligand, the Fas Ligand (CD-40 L). Now lung carcinoma cells have been shown to express Fas L, which probably enables them to induce apoptosis in cytotoxic T-lymphocytes and evade immunity [7]. The reverse situation– expression of the Fas L on the T cell and the Fas on lung cancer and other tumors (including B-cell malignancies) is also well known and there are therapeutic implications for treatment of such tumors with soluble form of Fas L, a form of immunotherapy [8].

TYPES OF IMMUNOTHERAPY

Active immuno-therapies for cancer directly stimulate the patient's own immune system to fight the tumor whereas passive immuno-therapies use components of the immune system such as antibodies created *in-vitro* to indirectly stimulate the immune attack. Broadly, there are three types of immuno-therapies and these are as follows:

(*i*) Cancer vaccines (active specific immuno-therapies)

(*ii*) Monoclonal antibody therapy (passive immuno-therapy (*Fig*. 3)

(*iii*) Nonspecific immunotherapies and adjuvants

(*i*) Cancer vaccines (active specific immuno-therapies)

A cancer vaccine contains cancer cells altered,

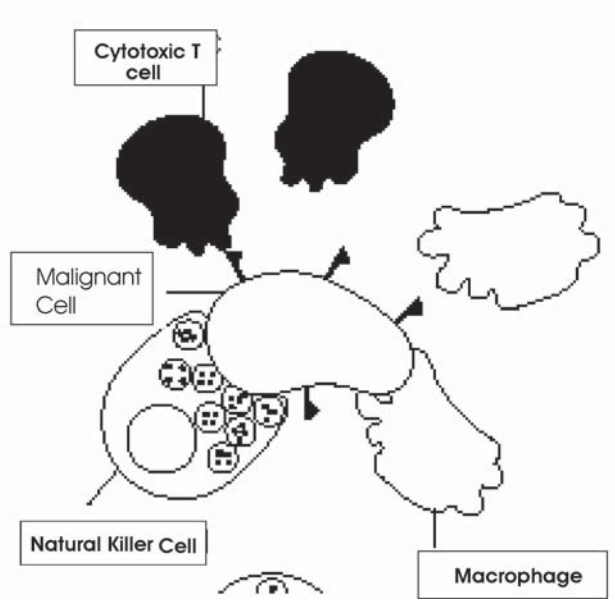

Fig. 2. Line diagram showing basic tumor immunology and immune response to cancer.

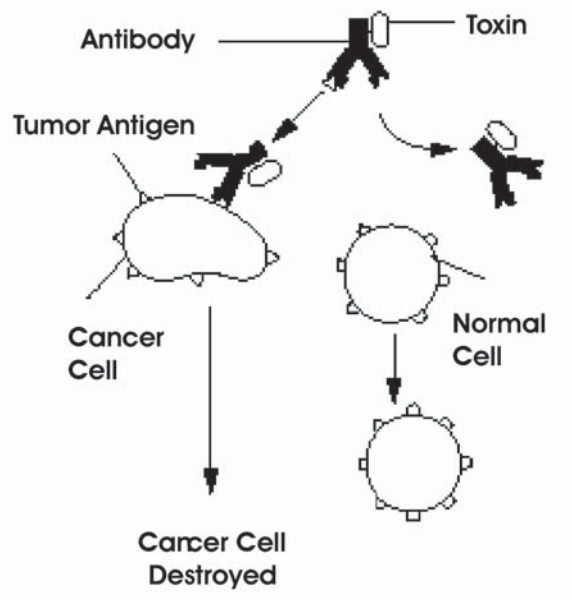

Fig. 3. Line diagram showing antibody linked to a toxin homing on to tumor surface and allowing toxin induced tumor cell destruction.

parts of cells, or pure antigens and because they do not bring about a generalized immune response, they are specifically targeted to the tumor. They work by inducing cell mediated and or humoral immunity.

Tumor cell vaccines: Autologous and allogeneic vaccines

Tumor cell vaccines use tumor tissue removed per-operatively and are then subjected to radiation. This is then injected back into the patient. Antigens on the tumor cells are still there, and so they stimulate a specific immune system response leading to recognition and attack by the immune system. The two basic kinds of tumor cell vaccines are autologous and allogeneic [9]. Trials of autologous cancer vaccines for clinical use in NSCLC have now become common since advances in molecular immunology have made it clear that NSCLC like SCLC is an immunogenic tumor and is amenable to immunotherapy [9].

Allogenic vaccines use cells of a particular tumor type that originally come from someone other than the treated patient. Sometimes a mixture of cells, originally removed from several patients is used for making a particular vaccine. The tumor cells are inactivated and are usually injected along with one or more adjuvants. Allogenic tumor cell vaccines are being studied in clinical trials against several types of malignancies, including melanoma, renal cancer, breast cancer, colorectal cancer, lung cancer and leukemia.

Dendritic cell vaccines

Dendritic cells are specialized antigen-presenting cells that process antigens on the tumor cell surfaces into peptides, and then present these peptides to T cells, making it easier for the immune system cells to recognize these and attack them. Dendritic cell vaccines, like autologous cell vaccines, are patient-specific and must be made individually for each patient. The process used to create them is complicated and expensive: (1) Dendritic cells are harvested from peripheral blood mononuclear cells (PBMCs) and then are stimulated. (2) These dendritic cells are pulsed with antigens in vitro, or are genetically modified so that they make their own antigens [10]. Some newer studies use dendritic cells fused with tumor cells (Fused Cells) creating dendritic cells with tumor antigens on their surface [11]. (3) The dendritic cells are then injected back into the body. (4) The "trained" dendritic cells are better able to help the immune system recognize and destroy tumor cells that have those antigens on them. (5) Furthermore, CD16 (Cluster of Differentiation 16) positive DCs derived from PBMCs can serve as better immune response enhancers as compared to CD16 negative DCs derived from PBMCs [12]. In a recent *in-vitro* study, peripheral blood monocyte derived DCs were co-cultured with primary lung cancer cells removed at surgery and then irradiated [13]. It was demonstrated that this *in-vitro* system was a superior DC based vaccine as compared to another DC based vaccine that used tumor cell lysate in the generation of antigen specific T-cell responses. The reason for the superior findings was that these DCs captured whole irradiated human lung cancer cells and were able to present a well defined surrogate antigens derived from these cells.

In another recent study, DC vaccines were generated from autologous CD14 positive PBMC precursors and were then delivered to 16 patients with stage IA to IIIB NSCLC also treated concurrently with standard multimodality surgery/chemo-radiation [14]. The DCs were initially pulsed ex-vivo with apoptotic bodies of allogenic lung cancer cell lines that over expressed HER2/neu, WT1, CEA, MAGE-2 and other tumor antigens. There was a measurable immune response to specific and non-specific tumor antigens in 11 patients. This vaccine was well tolerated and had biological and immunological activity.

The DC vaccine is offered through clinical trials to patients with prostate cancer, melanoma, renal carcinoma, colorectal cancer, lung cancer and non-Hodgkin's lymphoma [15].

Antigen vaccines

Antigen vaccines stimulate the immune system by using individual antigens, rather than whole tumor cells that contain several antigens. While antigen vaccines may be specific for a certain types of cancer, they are not made specifically for individual patients unlike autologous cell vaccines, and therefore can be mass-produced in the laboratory. This mass production allows large amounts of these very specific antigens to be made and for them to be used on several patients with a given tumor type.

There has also been a greater understanding of the genetic influences on antigen structure following the completion of the Human Genome project. The genetic codes of many cancer antigens have been

determined and serological analysis of recombinant cDNA expression libraries (SEREX) has led to the identification of many antigens recognized by the immune system of cancer patients (Cancer Immunome). Analysis of several antigens using bio-informatics and RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) has identified SLCO6A1 a gonad-specific antigen that is also expressed on lung cancer cell lines as a potential target for immune therapy [16]. These antigens can also be altered to make them more easily recognized by the immune system especially by the Major Histocompatibility (MHC) antigen. Identification of MHC class II–restricted tumor antigens which are capable of stimulating CD4+ T cells more effectively has now become possible [17]. CD4+ T cells release cytokines that enhance humoral (antibody related immunity) and so such studies [17] have generated interest on the role of humoral immunity in the treatment of lung cancer.

In a recent study, the immunological potential of putative tumor antigen SOX-4, which is a developmental transcription factor and is over-expressed on primary SCLC, was evaluated [18]. SOX-4 specific CD4 and CD8 positive T-cells were isolated from PBMCs of patients with SCLC which proves that there was a state of T-cell responsiveness to tumor antigen SOX-4 in these patients. When tested on PBMCs of healthy donors, no such responsiveness was found. The antigen specific immune responsiveness to SOX-4 in patients with SCLC supports the potential for its use as lung tumor vaccine [18].

MAGE-3 protein is a cancer testis antigen but is also expressed on NSCLC tumor cells. A study evaluating the CD4+T cell responsiveness to a protein MAGE -3 linked to an adjuvant AS02B was published recently [19]. Another example is that of tumor antigen WT1 (Wilm's Tumor 1 gene).This is over expressed in lung and breast cancer. Two patients with advanced lung cancer were immunized with WT1 peptide [20]. There was a reduction in tumor size and the levels of tumor markers.

A vaccine referred to as L-BIP25 (a vaccine that targets MUC-1 protein that is over expressed in NSCLC) improved survival and quality of life in patients with stage IIIB/ IV non small cell lung cancer. Interim results from this trial were discussed at the annual meeting of the European Society of Medical Oncology in 2004.

Long chain polysialic acid (Poly SA) conjugated to keyhole limpet hemocyanin was used as a peptide antigen on patients with SCLC who had successfully completed initial therapy with no apparent residual disease [21]. Measurable immune responses were obtained. In this study it was demonstrated that the N-propionylated-polySA antigen (NP-polySA) was superior to the polySA antigen in eliciting an immune response [21].

One of the reasons for the failure of peptide vaccines in clinical trials was the absence of a pre-vaccination strategy. There are clinical trials of peptide vaccines in lung cancer in which peptide-specific memory cyto-toxic T-Lymphocytes (CTLs) and their reactivity with a panel of known tumor antigens in individual patients with lung cancer is determined before vaccination. Only the antigens that show optimal reactivity with the CTLs before the vaccination are used for the final vaccination process [22]. Such trials with "CTL precursor-oriented peptide vaccinations" have also shown better clinical results in the form of longer progression free survival [22].

Antigen vaccines are being studied for use against breast cancer, prostate cancer, colorectal cancer, ovarian cancer, lung cancer, melanoma, and pancreatic cancer.

Anti-Idiotype vaccines

The unique part of each type of antibody is called an idiotype. The immune system also produces some antibodies that treat other antibodies like antigens. In other words, sometimes antibodies themselves act as antigens, triggering an immune response. Encouraging results have been obtained in recent clinical trials using these anti-idiotype antibodies as vaccines [23].

Anti-idiotypic antibody BEC-2 mimics structurally ganglioside GD3, which is expressed on the surface of most SCLC tumors (*Fig*. 4). In a clinical study [24], anti-idiotypic antibody BEC2 along with BCG (immune-adjuvant) was used as a vaccine on 15 patients with extensive Small Cell Lung Cancer (SCLC). They were then evaluated serologically for the presence of anti-BEC2 antibodies. All patients in this study developed anti-BEC2 antibodies and had an overall survival and relapse/progression free advantage [24]. Similarly, there are clinical studies which have used anti-idiotypic antibody N-acetyl or

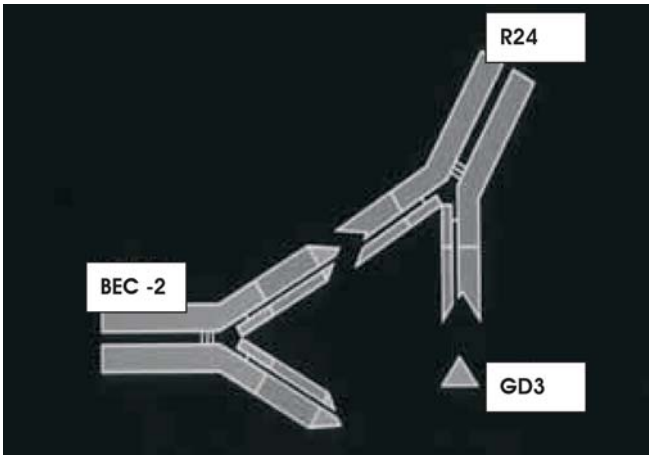

Fig. 4. Diagram showing the relation between the structures of GD3 (lung cancer antigen), R24 (antibody to GD3), and BEC-2, anti-idiotypic antibody (antibody to R24 antibody).

N-glycolylneuraminic acid GM3 ganglioside antigen as vaccine. These have shown encouraging results in lung cancer, breast cancer and melanoma [25,26]. A cancer vaccine named IGN101 which is a protein similar in structure to an antigen on the surface of epithelia of several cancers has been used in lung cancer patients. In Phase I and Phase II trials IGN101 has been shown to induce an immune response, even when administered under concomitant chemotherapy, and to reduce the number of disseminated tumor cells in peripheral blood. IGN101 is currently in a pivotal Phase II/III trial in non-small cell lung cancer (NSCLC).

DNA vaccines

When antigens or anti-idiotypes are injected into the body as a vaccine, they may produce the desired immune response at first but often become less effective over time and a state of immune-tolerance develops to the antigen/anti-idiotype. To get around this, there have been studies looking for ways to provide a steady supply of antigens to keep the immune response from waning and preventing immune-tolerance. Instead of the antigen segments of DNA, that could potentially be taken up by tumor cells and could instruct these tumor cells to continuously produce the DNA encoded antigens, are being used as immuno-stimulants. These therapies are called "DNA vaccines". DNA vaccines are now being studied in clinical trials for use against the following melanoma, leukemia, prostate cancer, lung cancer and head and neck cancer.

A study using a DNA vaccine encoding human carcinoembryonic antigen (CEA) which activates CTLs and DCs was undertaken in CEA transgenic mice [27]. Eradication of subcutaneous tumors and prevention of pulmonary metastases was shown suggesting that this approach could be an effective new treatment modality for human lung cancer.

Other active specific immunotherapies

Lymphokine-Activated Killer (LAK) Cell Therapy: A large numbers of active, cytoxic T-cells can be produced in the lab by treating a small number of T- cells *in-vitro* with interleukin-2 (IL-2) and culturing them. After being returned to the patient's blood-stream, these special cells, called lymphokine-activated killer (LAK) cells, are more effective against cancer cells. Researchers are currently testing several ways to use these very active forms of immuno-therapy. LAK cell therapy has shown promising results in animal studies, where it brought about shrinkage of tumors in animals with lung, liver, and other cancers. While clinical trials in human patients have not yet produced results as successful as those in animals, researchers are constantly improving LAK cell techniques. They are testing these newly improved methods against melanoma, brain tumors, and other cancers.

Tumor-Infiltrating lymphocyte (TIL) vaccine with interleukin-2 (IL-2)

Autologous lymphocytes teased out of tumor tissues either post-operatively or by fine needle aspiration are called "Tumor Infiltrating Lympho-cytes". These cells can be removed from tumor samples taken from a patient and forced to reproduce *in-vitro* by treating them with IL-2. When re-introduced into the patient, these cells may be immunologically active.

One research group has isolated several cytotoxic T-Lymphocyte (CTL) clones from a patient with lung cancer [28]. It was found that these CTL clones were selectively expanded in vivo at the tumor site as compared to the peripheral blood indicating that immune responses by these effector T-cells may be contributing to tumor regression in this patient with NSCLC [28].

Immunotherapies using TILs are being tested in clinical trials for people with melanoma, ovarian cancer, lung cancer and other cancers.

(*ii*) Monoclonal antibodies

Monoclonal antibodies (*Fig*. 4) achieve their therapeutic effect through various mechanisms. They can have direct effects in producing apoptosis or programmed cell death. They can block growth factor receptors, effectively arresting proliferation of tumor cells.

Indirect effects include recruiting cells that have cytotoxic effect, such as monocytes, macrophages and Natural Killer Cells [4]. Effector cells, such as Natural Killer (NK) cells, T- and B-lymphocytes, macrophages, dendritic cells and neutrophils, are present either within or around tumours and are likely to play a role in cancer only in conjunction with humoral immunity (antibody immunity).

Another type of antibody-mediated tumor cell kill is called antibody-dependent cell mediated cytotoxicity (ADCC) [29]. Monoclonal antibodies (MoAbs) also bind complement, leading to direct cell toxicity, known as complement dependent cytotoxicity (CDC). The mechanism involved in ADCC tumor cell kill is as follows: the Natural Killer cells have IgG receptors (Fc gamma RIII) on their surface and are therefore able to home onto the tumor/ virally infected cells and cause cytotoxicity on target tissue non-specifically [4]. A similar mechanism may be involved in the damage to endothelial cells in autoimmune disease [29] and has potential for use as angiogenesis inhibition. MoAbs are also used in therapeutic clinical trials as: (*i*) mediators of humoral immunity; (*ii*) carriers of cytotoxic agents-by linking them to toxins (*Fig*. 5); (*iii*) agents to block tumour growth factors or angiogenesis; or (*iv*) anti-idiotypic vaccines.

A complementarity determining region grafted humanized antibody (sibrotuzumab) that is directed against human fibroblast activation protein (FAP) has been evaluated in an open-label dose escalation study which also had 6 patients with non-small cell lung cancer. It was found to be safe and well tolerated although no objective responses were demonstrable. Therefore it was also concluded that sibrotuzumab can be administered safely to patients with advanced FAP-positive cancer including lung cancer [30].

(*iii*) Non specific immunotherapies including cytokines and adjuvants

Advances in molecular biology and discovery of

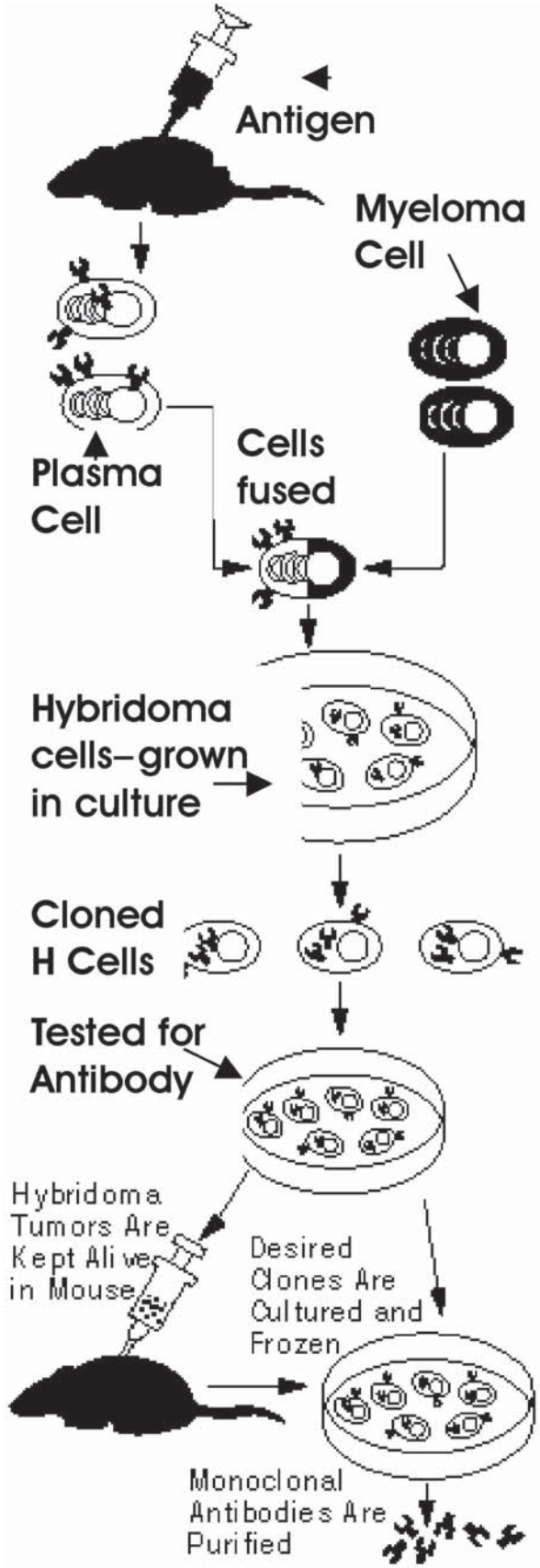

Fig. 5. Line diagram showing methodology of monoclonal antibody production.

the Tyrosine Kinase inhibitors is one of the landmarks in the development of therapy of cancer especially lung cancer. Although not strictly a form of immunotherapy, these are being increasingly used along with immunotherapy with good results.

An example of a successful, bi-specific inhibitor is a molecule directed to HER1/EGFR Tyrosine Kinase receptor (Erlotinib) [31]. After its establishment as safe therapy for NSCLC, it has been used in combination with Bevacizumab, which is an angiogenesis inhibitor antibody, in phase II clinical trials for patients with NSCLC [32]. Gefitinib is another HER1/EGFR Tyrosine Kinase inhibitor that has been approved for refractory lung cancer [33].

IL-12 is a cytokine with immune modulating functions. Administration of IL-12 to tumor-bearing mice has resulted in tumor regression through mechanisms involving efficient IFN-gamma production by anti-tumor T-cells at tumor sites in situ and the establishment of a tumor-specific protective immune response. This indicates that IL-12 can induce a curative immune response in the face of an aggressive micrometastasizing tumor [34].

In the future, the shortcomings of immunotherapy maybe overcome with concomitant use of gene therapy. An example of combined immunotherapy and gene therapy for advanced NSCLC is the use of a recombinant MVA (Modified Vaccinia Ankara) expressing MUC1 protein and IL-2 (TG4010). Interim results from this phase to study were encouraging [35].

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