IMMUNOLOGY - CHAPTER EIGHTEEN

TUMOR IMMUNOLOGY

MALIGNANT TRANSFORMATION

The proliferation of normal cells is carefully regulated. However, such cells when exposed to chemical carcinogens, irradiation and certain viruses may undergo mutations leading to their transformation into cells that are capable of uncontrolled growth, producing a tumor or neoplasm.

A tumor may be:

- Benign, if it is not capable of indefinite growth and the host survives.
- Malignant, if the tumor continues to grow indefinitely and spreads (metastasizes), eventually killing the host. This uncontrolled growth may be due to up regulation of oncogenes (cancer-inducing genes) and/or down regulation of tumor suppressor genes (that normally inhibit tumor growth often by inducing cell death).

EVIDENCE FOR IMMUNE REACTIVITY TO TUMORS

There is a lot of evidence that tumors can elicit an immune response. Such evidence includes:

- Tumors that have severe mononuclear cell infiltration have a better prognosis than those that lack it.
- Certain tumors regress spontaneously (e.g., melanomas, neuroblastomas), suggesting an immunological response.
- Some tumor metastases regress after removal of primary tumor which reduces the tumor load, thereby inducing the immune system to kill the residual tumor.
- Although chemotherapy leads to rejection of a large number of tumor cells, the few tumor cells that evade the action of the drugs can outgrow and kill the host. However, the immune system may be able to mount an attack against the few tumor cells that are spared by the chemotherapeutic agent.
- There is an increased incidence of malignancies in immuno-deficient patients such as AIDS patients who are susceptible to Kaposi sarcoma and transplant patients who are susceptible to Epstein Barr virus (EBV)-induced lymphoma.
- Tumor-specific antibodies and T lymphocytes (detected in cytotoxicity and proliferative response assays) have been observed in patients with tumors.
- The young and the old population have an increased incidence of tumors. These members of the population often have an immune system that is compromised.
- Hosts can be specifically immunized against various types of tumors demonstrating tumor antigens can elicit an immune response.

TUMOR ASSOCIATED ANTIGENS

In order for the immune system to react against a tumor, the latter must have antigens that are recognized as foreign. A number of alterations in gene expression occur in cells during tumorigenesis. Tumorigenesis may lead to expression of new antigens (neoantigens) or alteration in existing antigens that are found on normal cells. These antigens may include membrane receptors, regulators of cell cycle and apoptosis, or molecules involved in signal transduction pathways.

There are 2 main types of tumor antigens:

- Tumor-specific transplantation antigens (TSTA) which are unique to tumor cells and not expressed on normal cells. They are responsible for rejection of the tumor.
- Tumor associated transplantation antigens (TATA) that are expressed by tumor cells and normal cells.

Although chemical- , UV- or virus-induced tumors express neo-antigens, the majority of these tumors are often weakly immunogenic or non-immunogenic. In most cases, TSTAs cannot be

identified easily. Some of these antigens may be secreted while others may be membrane-associated molecules.

Tumor associated transplantation antigens (TATA)
The majority of tumor antigens are also present on normal cells and are referred to as tumor associated transplantation antigens. They may be expressed at higher levels on tumor cells when compared to normal cells. Alternatively, they may be expressed only during development of cells and lost during adult life but re-expressed in tumors.

Tumor-associated developmental antigens or onco-fetal antigens
These include alpha-fetoprotein (AFP) and carcino-embryonic antigen (CEA) found secreted in the serum. AFP is found in patients with hepatocellular carcinoma whereas CEA is found in colon cancer. These are important in diagnosis.AFP is produced only as a secreted protein whereas CEA is found both on cell membranes and in secreted fluids. Since secreted antigens contribute little toward immunity against tumors, the role of these neo-antigens in immuno-surveillance is questionable.

The normal range of AFP concentrations in humans is 0-20 ng/ml. This level rises considerably in patients with hepatomas and non-seminal testicular carcinoma. A 5-fold or higher rise in this protein is used for monitoring hepatomas and testicular cancers. AFP level may also be raised in some non-malignant conditions, such as cirrhosis, in hepatitis and other forms of liver damage.

CEA levels in normal people range up to 2.5 ng/ml, but they increase significantly in certain malignancies, particularly colo-rectal cancers. They may also rise in some non-malignant conditions (such as chronic cirrhosis, pulmonary emphysema and heavy smoking). Levels that are 4 to 5 times normal have been used to predict recurrence of colo-rectal tumors.

TUMOR ASSOCIATED TRANSPLANTATION ANTIGENS ON VIRAL TUMORS

Viruses that cause human tumors include:

DNA viruses

- Papova (papilloma, polyoma) viruses: Papilloma virus causes cervical cancer.
- Hepatitis virus: Hepatitis B virus causes hepatocellular cancer.
- Adenoviruses may also be tumorigenic

RNA viruses

- Retroviruses: Human T-lymphotropic viruses (HTLV-I and HTLV-II) causes T cell leukemias.

A number of viruses cause different types of tumors in animals (for example, SV-40 virus,

adenovirus, Rous sarcoma virus, Friend erythroleukemic virus, Moloney Rauscher and Gross viruses). Viruses are involved or suspected to be involved in some human malignancies (HTLV-1 in leukemia, hepatitis-B virus in hepatic carcinoma, papilloma virus in cervical cancer). Virus-induced tumors express cell surface antigens (distinct from antigens of the virion itself) which are shared by all tumors induced by the same virus. These antigens are characteristic of the tumor-inducing virus, regardless of tissue origin of the tumor or animal species in which the tumor exists (Figure 1). More information on tumor viruses can be found in the section on oncogenic viruses

TUMOR ASSOCIATED TRANSPLANTATION ANTIGENS ON CHEMICALLY-INDUCED TUMORS

Chemically-induced tumors are different from virally-induced tumors in that they are extremely heterogeneous in their antigenic characteristics. Thus, any two tumors induced by the same chemical, even in the same animal, rarely share common tumor specific antigens (Figure 2). These unique antigens on chemically-induced tumors are referred to as tumor specific transplantation antigens (TSTA).

SYNGENEIC, ALLOGENEIC AND XENOGENEIC TUMORS

A tumor that grows in an animal strain will also grow in another animal belonging to the same inbred strain obtained by repeated brother-sister matings. These animals express the same MHC molecules and are referred to as *syngeneic*. However, most normal animal populations are *allogeneic* and have various MHC haplotypes. Thus, a tumor transferred from one animal to another animal belonging to an outbred strain is rejected because of the allo-MHC rather than the TSTA. A tumor transferred from an animal belonging to one species to another animal belonging to a different species is rapidly rejected because the animals are *xenogeneic*.

IMMUNITY AGAINST TUMORS

Although there is ample evidence for anti-tumor immune reactivity in humans, evidence for immunity against malignancy comes mostly from experimental studies with animals. In these, mice were immunized by administering irradiated tumor cells or following removal of a primary tumor challenged with the same live tumor. These animals were found to be resistant to rechallenge with the same live tumor. While antibodies may develop against few cancers, cell-mediated immunity plays a critical role in tumor rejection. Thus, immunity can be transferred, in most cases, from an animal, in which a tumor has regressed, to a naive syngeneic recipient by administration of T lymphocytes. The T helper (Th) cells recognize the tumor antigens that may be shed from tumors and internalized, processed and presented in association with class II MHC on antigen presenting cells. These Th cells, when activated, will produce cytokines. Thus, the Th cells provide help to B cells in antibody production.

Cytokines such as IFN-gamma may also activate macrophages to be tumoricidal. Furthermore, the Th cells also provide help to tumor-specific cytotoxic T cells (CTLs) by inducing their proliferation and differentiation. The CTLs recognize tumor antigens in the context of class I MHC and mediate tumor cell lysis. In tumors that exhibit decreased MHC antigens, natural killer (NK) cells are important in mediating tumor rejection.

ESCAPE FROM IMMUNO-SURVEILLANCE

According to the Immune Surveillance Theory, cancer cells that arise in the body are eliminated by the immune system. However, due to impaired immune reactivity, cancer cells may escape destruction. Tumors evade immune recognition by several mechanisms. Tumors may not express neo-antigens that are immunogenic or they may fail to express co-stimulatory molecules required for the activation of T cells. In addition, certain tumors are known to lack or be poor expressers of MHC antigen. Another reason for failure of immune surveillance may be the fact that in the early development of a tumor, the amount of antigen may be too small to stimulate the immune system (low dose tolerance) or, due to the rapid proliferation of malignant cells (high dose tolerance), the immune system is quickly overwhelmed. In addition, some tumors may evade the immune system by secreting immunosuppressive molecules and others may induce regulatory cells particularly the CD4+CD25+ FoxP3+ T regulatory cells. Also, some tumors may shed their antigens which in turn may interact and block antibodies and T cells from reacting with the tumor cells.

USE OF TUMOR NEO-ANTIGENS IN PATIENT MANAGEMENT

The presence of neo-antigens on tumor cells has been exploited for both diagnostic and therapeutic purposes.

Immuno-diagnosis

Monoclonal antibodies labeled with radioisotope have been used for *in vivo* detection of relatively small tumor foci. Antibodies have also been used *in vitro* to identify the cell origin of undifferentiated tumors, particularly of lymphocytic origin. Also, immuno-histological staining is used to confirm suspected metastatic foci, especially in bone marrow.

Immunotherapy

Immunotherapy has been used as a novel means of treating cancer. Both active and passive means of stimulating the non-specific and specific immune systems have been employed, in some cases with significant success.

- **Active** **Immunotherapy**

In this, the host actively participates in mounting an immune response

- Specific activation is achieved by using vaccines: e.g. Hepatitis B vaccine and Human Papilloma virus (HPV) vaccine

- Non-specific activation is achieved by immunization with, for example, Bacillus Calmette-Guerin (BCG) and Corynebacterium parvum

These activate macrophages to be tumoricidal.

- **Passive Immunotherapy**
  This involves transfer of preformed antibodies, immune cells and other factors into the hosts.

  - Specific:

    i) Antibodies against tumor antigens (e.g. Her2/Neu for treatment of breast cancer)

    ii) Antibodies against IL-2R for Human T lymphotropic virus (HTLV-1)-induced adult T cell leukemia

    iii) Antibodies against CD20 expressed on non-Hodgkin's B cell lymphoma. These antibodies bind to tumor antigens on the cell surface and activate complement (C') to mediate tumor cell lysis. In addition, Fc receptor bearing cells such as NK cells, macrophages and granulocytes may bind to the antigen-antibody complexes on the tumor cell surface and mediate tumor cell killing through antibody-dependent cell-mediated cytotoxicity.

    iv) Antibodies conjugated to toxins, radioisotopes and anti-cancer drugs have also been used. These enter the cells and inhibit protein synthesis. e.g. anti-CD20 conjugated to Pseudomonas toxin or ricin toxin.

There are several problems with the use of antibodies

- Antibodies are not efficient because the tumor antigens are associated with class I MHC antigens.
- Tumors may shed antigen or antigen-antibody complexes. Thus, immune cells cannot mediate tumor destruction.
- Some antibodies may not be cytotoxic.
- Antibodies may bind non-specifically to immune cells expressing the Fc receptors which include NK cells, B cells,

macrophages and granulocytes without binding to tumor cells.

- Nonspecific:

i) Adoptive Transfer of lymphocytes:

- Lymphokine-activated killer (LAK) cells which are IL-2 activated T and NK cells.
- Tumor-infiltrating lymphocytes (TIL)

ii) Dendritic cells pulsed with tumor antigens may induce tumor-specific T cell responses. As tumor Ags are usually not known, tumor lysates are used.

iii) Cytokines

- IL-2: Activates T cells/NK cells expressing IL-2 receptors. This is used in the treatment of renal cell carcinoma and melanoma
- IFN-alpha: This induces MHC expression on tumors and used in the treatment of hairy B cell leukemias
- IFN-gamma: This increases class II MHC expression; used in the treatment of ovarian cancers.
- TNF-alpha: This kills tumor cells.

iv) Cytokine gene transfected tumor cells may also be used which can activate T or LAK cell-mediated anti-tumor immunity.

Table 1. Immunotherapy of tumors

Active	**Non-specific**	BCG, *Propionibacterium acnes*, levamisole, cytokine genes, *etc*.
	Specific	Killed tumor cells or their extract, recombinant antigens, idiotype,

Passive	**Nonspecific**	co-stimulatory molecule genes, *etc*. LAK cells, cytokines
	Specific **Combined**	Antibodies alone or coupled to drugs, pro-drug toxins or radioisotope; bispecific antibodies; T-cells LAK cells and bispecific antibody
* BCG: Bacillus Calmette Geurin is a bovine strain of *Mycobacterium tuberculosis*		

A variety of immunopotentiating agents (biological response modifiers) are used to enhance anti-tumor immunity. They include bacterial products, synthetic chemicals and cytokines (Table 2). Most of these agents exert their effects by activating macrophages and natural killer (NK) cells, eliciting cytokines or enhancing T-cell functions.

Table 2. Non-specific active immunotherapy: biological response modifiers (BRMs)

Type of BRM	Examples	Major effect
Bacterial product	BCG, *P. acnes*, muramyl di-peptide, trehalose dimycolate	Activate macrophages and NK cells (via cytokines)
Synthetic molecules	Pyran, poly I:C, pyrimidines	Induce interferon production
Cytokines	Interferon-alpha -	Activate macrophages

	beta, - gamma, IL-2, TNF	and NK cells

A number of cytokines have been used to potentiate the immune function of the host since the discovery that these cytokines have potent and selective effects on certain components of the immune system (Table 3).

Table 3. Cytokine therapy of tumors

Cytokine	Tumor type and result	Anti-tumor mechanism(s)
IFN-alpha, beta	Remission of hairy cell leukemia, weak effect on some carcinomas	Increased expression of class I MHC, possible cytostatic anti-tumor effect,
IFN-gamma	Remission of peritoneal carcinoma of ovary: ineffective systemically	Increased MHC antigens; macrophage, Tc and NK cell activation
IL-2	Remission in renal carcinoma and melanoma	T-cell proliferation and activation, NK cells activation
TNF-alpha	Can reduce malignant ascites	Macrophage and lymphocyte activation

Monoclonal anti-tumor antibodies have been used in different forms for the treatment of cancer, either because of their direct effect or as vehicles to target anti-cancer drugs, toxins

and the non-specific components of the host's immune system to the site of tumor (Figure 3). In addition, such specific antibodies are also used in the diagnosis of metastatic lesions, otherwise not detectable by conventional radiologic means.