TUMOR IMMUNOLOGY

Teaching Objectives:
Introduction to Cancer Immunology
Know the antigens expressed by cancer cells
Understand the nature of immune response to tumors
Study how cancers evade immune system
Describe the approaches used in Immunotherapy

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

1) Benign, if it is not capable of indefinite growth and the host survives.
2) Malignant, if the tumor continues to grow indefinitely and spreads (metastasizes), eventually killing the host.

This uncontrolled growth may be due to upregulation of **oncogenes** (cancer-inducing genes) and/or downregulation of **tumor suppressor genes** (that normally inhibit tumor growth often by inducing cell death).

Evidence for existence of an immune response against tumors
The following criteria serve as evidence that tumors can elicit an immune response.

1. Tumors that have severe mononuclear cell infiltration have a better prognosis than those that lack it.
2. Certain tumors regress spontaneously (*e.g.*, melanomas, neuroblastomas).suggesting an immunological response.
3. Some tumor metastases regress after removal of primary tumor which reduces the tumor load, thereby inducing the immune system to kill the residual tumor..
4. 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.
5. There is an increased incidence of malignancies in immunodeficient patients such as AIDS patients who are susceptible to Kaposi sarcoma and transplant patients who are susceptible to Epstein Barr virus (EBV)-induced lymphoma.
6. Tumor-specific antibodies and T lymphocytes (detected in cytotoxicity and proliferative response assays) have been observed in patients with tumors.
7. 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.
8. Hosts can be specifically immunized against various types of tumors demonstrating tumor Ags can elicit an immune response.

Tumor antigens

Tumorigenesis may lead to expression of new antigens 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.

1. **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.
2. **Tumor associated transplantation antigens** (TATA) that are expressed by tumor cells and normal cells.

Although chemical- , UV- or virus-induced tumors express **neo-antigens,** majority of the tumors are often weakly immunogenic or non-immunogenic. In most cases, tumor-specific transplantation Ags cannot be identified easily. Also, some of these antigens may be secreted while others include membrane-associated molecules.

Tumor associated transplantation antigens (TATA)

The majority of tumor Ags are also present on normal cells and are referred to as **tumor associated transplantation antigens** (**TATA**). 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 Ags 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.

Virus-induced tumors:

Viruses that cause tumors include

DNA viruses:

1. Papova (papilloma, polyoma) viruses. Ex. Papilloma virus causes cervical cancer.
2. Hepatitis virus: Hepatitis B virus causes hepatocellular cancer.
3. Adenoviruses

RNA viruses:

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

Virus-induced tumors express **tumor-associated viral Ags**. These are cell surface antigens that are distinct from antigens on the virion itself. However, these transplantation-associated viral Ags are shared by all tumors induced by the same virus, regardless of tissue origin of the tumor or animal in which the tumor exists.

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. 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**.

Immune response to tumors:
Evidence for immunity against malignancy comes mostly from experimental studies, wherein 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 Abs 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 Ags 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 Ab production. The cytokines such as IFN-γ may also activate macrophages to be tumoricidal. Furthermore, the Th cells also provide help to tumor-specific cytotoxic T cell (CTL) by inducing their proliferation and differentiation. The CTLs recognize tumor Ags in the context of class I MHC and mediate tumor cell lysis. In tumors that exhibit decreased MHC Ags, natural killer (NK) cells are important in mediating tumor rejection.

How tumors evade immune system:
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, the cancer cells 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.

Immunotherapy

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

1) Active Immunotherapy: Wherein the host actively participates in mounting an immune response
   a) Nonspecific activation is achieved by immunization with:
      i) Bacillus Calmette-Guerin (BCG)
      ii) Corynebacterium parvum
      These activate macrophages to be tumoricidal.
   b) Specific activation using vaccines:
      i) Hepatitis B vaccine
      ii) Human Papilloma virus (HPV) vaccine

2) Passive Immunotherapy: This involves transfer of preformed Abs, immune cells and other factors into the hosts.
   a) Specific:
      i) Antibodies against tumor Ags (e.g. Her2/Neu for treatment of breast cancer)
      ii) Abs against IL-2R for Human T lymphotropic virus (HTLV-1)-induced adult T cell leukemia
      iii) Abs against CD20 expressed on non Hodgkin's B cell lymphoma.

      These Abs bind to tumor Ags 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 Ag-Ab complexes on tumor cell surface and mediate tumor cell killing through Ab-dependent cell-mediated cytotoxicity.

      iv) Abs 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 Abs
      1) Abs are not efficient because the tumor Ags are associated with class I MHC Ags.
      2) The tumors may shed Ag or Ag-Ab complexes. Thus, immune cells cannot mediate tumor destruction.
      3) Some Abs may not be cytotoxic.
      4) Abs may bind nonspecifically to immune cells expressing the Fc receptors which include NK cells, B cells, macrophages and granulocytes without binding to tumor cells.

b) Nonspecific:
   i) Adoptive Transfer of lymphocytes:
      (1) Lymphokine-activated killer (LAK) cells which are IL-2 activated T and NK cells.
      (2) Tumor-infiltrating lymphocytes (TIL)

   ii) Dendritic cells pulsed with tumor Ags may induce tumor-specific T cell responses. As tumor Ags are usually not known, tumor lysates are used.
   iii) Cytokines
      (1) IL-2: Activates T cells/NK cells expressing IL-2 receptors. Used in the treatment of renal cell carcinoma and melanoma,
      (2) IFNα: Induces MHC expression on tumors and used in the treatment of hairy B cell leukemias
      (3) IFN-γ: Increases class II MHC expression; used in the treatment of ovarian cancers.
      (4) TNF-α: Kills tumor cells.
   iv) Cytokine gene transfected tumor cells may also be used which can activate T or LAK cell-mediated anti-tumor immunity.

Immunization

TEACHING OBJECTIVES:

1. Know the distinction between passive and active immunization and their examples
2. Distinguish between artificial and natural means of immunization
3. Know the applications and problems of artificial passive immunization
4. Know the applications and problems of artificial active immunization
5. Know the modern approaches to immunization

Immunization is the means of providing specific protection against most common and damaging pathogens. The mechanism of immunity depends on the site of the pathogen and also the mechanism of it pathogenesis. Thus, if the mechanism of pathogenesis involves exotoxins, the only immune mechanism effective against it would be neutralizing antibodies that would prevent its binding to the appropriate receptor and promoting its clearance and degradation by phagocytes. Alternatively, if the pathogen produces disease by other means, the antibody will have to react with the organism and eliminate by complement-mediated lysis or phagocytosis and intracellular killing. However, if the organism is localized intracellularly, it will not be accessible to antibodies while it remains inside and the cell harboring it will have to be destroyed and, only then antibody can have any effect. Most viral infections and intracellular bacteria and protozoa are examples of such pathogens. In this case, the harboring cells can be destroyed by elements of cell mediated immunity or if they cause the infected cell to express unique antigens recognizable by antibody, antibody-dependent and complement mediated killing can expose the organism to elements of humoral immunity. Alternatively, cells harboring intracellular pathogen themselves can be activated to kill the organism. Such is the case with pathogens that have the capability of surviving within phagocytic cells.

Specific immunity can result from either passive or active immunization and both modes of immunization can occur by natural or artificial processes (Figure 1).

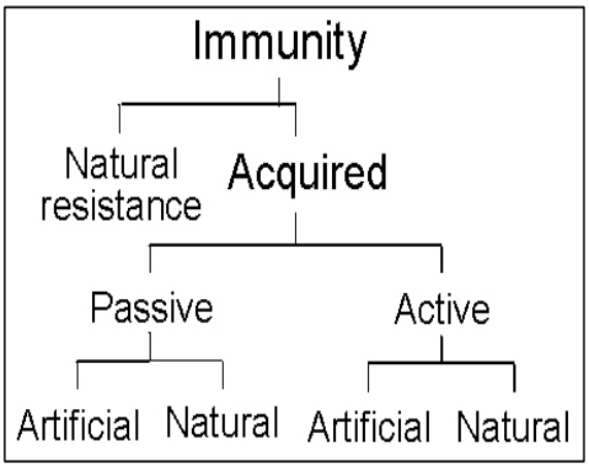

Figure 1 Different modes of acquiring immunity

Passive Immunity:

Immunity can be gained, without the immune system being challenged with an antigen, by transfer of serum or gamma globulins from an immune donor to a non-immune individual. Alternatively, immune cells from an immunized individual may be used to transfer immunity. Passive immunity may be acquired naturally or artificially.

Naturally acquired passive immunity: Immunity is transferred from mother to fetus through placental transfer of IgG or colostral transfer of IgA.

Artificially acquired passive immunity: Immunity is often artificially transferred by injection with gamma globulin from other individuals or from an immune animal. Passive transfer of immunity with immune globulin or gamma globulin is practiced in numerous acute infections (diphtheria, tetanus, measles, rabies, etc.), poisoning (insect-, reptile-bites, botulism), and as a

prophylactic measure (**hypogammaglobulinemia**). In these situations, gamma globulin of human origin is preferable although specific antibodies raised in other species (usually horse) are effective and used in some cases (*e*.g., poisoning, diphtheria, tetanus, gas gangrene, botulism, *etc*.). While this form of immunization has the advantage of providing immediate protection, it is effective for a short duration only and often results in pathological complications, such as **serum sickness** characterized by rash, fever, arthralgia, vasculitis, nephritis, *etc*., and anaphylaxis. Homologous immunoglobulin may carry the risk of transmitting hepatitis and HIV and other blood borne diseases.

Passive transfer of cell-mediated immunity (immunity that is transferred by cells and not by antibody) can also be accomplished in certain diseases (cancer, immunodeficiency). However, it is difficult to find histocompatible (matched) donors and there is severe risk of graft versus host disease.

Active Immunity:

This refers to immunity produced by the body following exposure to antigens.

Naturally acquired active immunity: Exposure to different pathogens leads to sub clinical or clinical infections, which normally result in a protective immune response against these pathogens.

Artificially acquired active immunity: Immunization may be achieved by administering live or dead pathogens or their components. Vaccines used for active immunization consist of live (attenuated: capable of producing very mild or no symptoms) organism, killed whole organism, microbial components or secreted, detoxified toxins (toxoid).

Live vaccines: Live organisms are used for immunization against a number of viral infections. Live vaccines for measles, mumps, rubella and chicken pox (varicella) are used routinely. A live bacterial vaccine consisting of a strain of *Mycobacterium bovis*, Bacillus Calmet Geurin (BCG) is used against tuberculosis in many African, European and Asian countries but not many others.

Whereas many studies have shown the efficacy of BCG vaccine, a number of studies also cast doubt on its benefits.

Live vaccines normally produce self-limiting non-clinical infections and lead to subsequent immunity, both humoral and cell-mediated, the latter being essential for intracellular pathogens. However, they carry a serious risk of **causing overt disease in immunocompromised individuals**. Furthermore, since live vaccines are often attenuated (made less pathogenic) by passage in animal or thermal mutation, they can revert to their pathogenic form and cause serious illness. It is for this reason, polio live (Sabin) vaccine, which was used for many years, has been replaced in many countries by the inactivated (Salk) vaccine.

Killed vaccines: These consist of whole organisms inactivated by heat, chemicals or UV irradiation treatment. Many killed viral and bacterial vaccines are available. Some of these are used to immunize people at risks (*e.g.* influenza, hepatitis A, etc.) while others are used to immunize travelers to different countries (*e.g.* cholera, typhoid *etc.*). Pertussis (whooping cough) whole bacterial vaccine was used routinely until a few years ago, but due to its serious side effect, it has been replaced by a formulation of acellular components.

Sub-unit vaccines: Some vaccines consist of subcomponents of the pathogenic organisms, usually proteins or polysaccharides. Since polysaccharides are relatively weak T-independent antigens, and produce only IgM responses without immunologic memory, they are made more immunogenic and T-dependent by conjugation with proteins (*e.g.*, haemophilus, meningococcus, pneumococcus, etc.). Hepatitis-B, rabies vaccines consist of antigenic proteins cloned into a suitable vector (*e.g.*, yeast). These subunit vaccines are designed to reduce the problems of toxicity and risk of infection. When the pathogenic mechanism of an agent involves a toxin, a modified form of the toxin (toxoid) is used as vaccine (*e.g.*, diphtheria, tetanus, *etc.*). Toxoids, although lose their toxicity, they remains immunogenic.

Other novel vaccines: A number of novel approaches to active immunization are in the investigative stage and are used only experimentally. These include anti-idiotype antibodies, DNA vaccines and immunodominant peptides (recognized by the MHC molecules) and may be available in the future. Anti-idiotype antibodies against polysaccharide antibody produce long lasting immune responses with immunologic memory. Viral peptide genes cloned into vectors, when injected transfect host cells and consequently produce a response similar to that produced against live-attenuated viruses (both cell-mediated and humoral). Immunodominant peptides are simple and easy to prepare and, when incorporated into MHC polymers, can provoke both humoral and cell mediated responses.

Adjuvants: Weaker antigens may be rendered more immunogenic by the addition of other chemicals. Such chemicals are known as **adjuvants**. There are many biological and chemical substances that have been used in experimental conditions (Table 1). However, only Aluminum salts (alum) are approved for human use and it is incorporated in DTP vaccine. Furthermore, pertussis itself has adjuvant effects. Adjuvants used experimentally include mixtures of oil and detergents, with (Freund's complete adjuvant) or without certain bacteria (Freund's incomplete adjuvant). Bacteria most often used in an adjuvant are Mycobacteria (BCG) and *Nocardia*. In some instance sub-cellular fractions of these bacteria can also be used effectively as adjuvants.

Newer adjuvant formulations include synthetic polymers and oligonucleotides. Most adjuvants recognize TOLL-like receptors thus activating mononuclear phagocytes and inducing selective cytokines that can enhance Th1 or Th2 responses, depending on the nature of the adjuvant.

Table 1. Selected adjuvants in clinical or experimental use

Adjuvant type	human use	Experimental only
Salts:		
aluminum hydroxide,	Yes	Slow release of antigen, TLR interaction and cytokine induction
aluminum phosphate-calcium phosphate	Yes	
Beryllium hydroxide	No	
Synthetic particles:		
Liposomes, ISCOMs,	No	Slow release of antigen
polylactates	No	
Polynucleotides:	No*	TLR interaction and cytokine induction
CpG and others		
Bacterial products:	Yes	TLR interaction and cytokine induction
B.pertussis	No	
M. bovis (BCG and others)	No	Antigen depot
Mineral oils		
Cytokines:	No*	Activation and differentiation of T- and B cells and APC.
IL-1, IL-2, IL12, IFN-γ, *etc*.		

*Experimental use in human malignancies

The protective immunity conferred by a vaccine may be life-long (measles, mumps, rubella, small pox, tuberculosis, yellow fever, etc.) or may last as little as a few months (cholera). The primary immunization may be given at the age of 2-3 months (diphtheria, pertussis, tetanus,

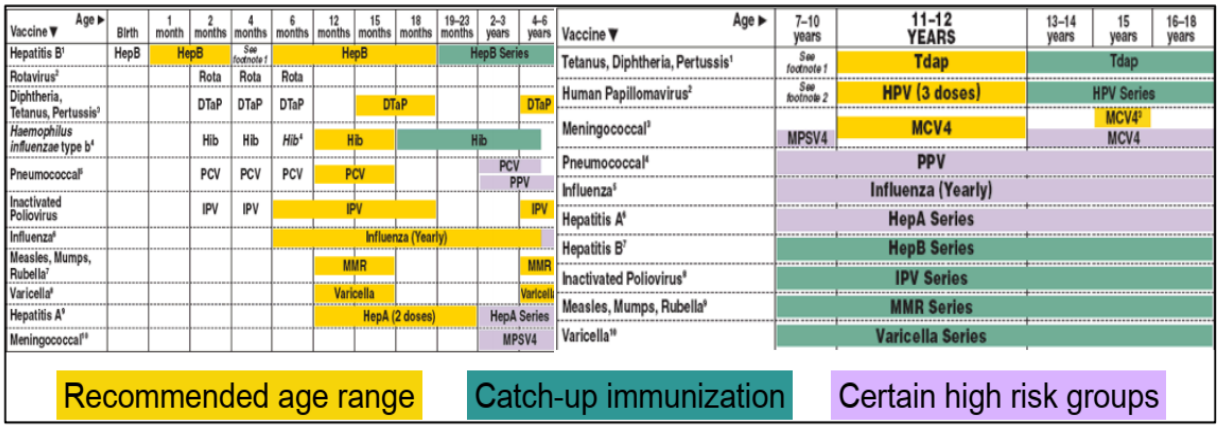

Figure 2. Recommended immunization schedule

polio), or 13-15 months (mumps, measles, rubella). The currently recommended schedule of routine immunization in the USA (recommended by CDC and AIP) is summarized in Figure 2. This schedule is revised on yearly basis or as need by the CDC Advisory Committee on Immunization Practice (AICP).

Prophylactic versus therapeutic immunization: Most vaccines are given prophylactic ally, *i.e.*, prior to exposure to the pathogen. However, some vaccines can be administered therapeutically , *i.e.*, post exposure (*e.g.*, rabies virus). The effectiveness of this mode of immunization depends on the rate of replication of the pathogen, incubation period and pathogenic mechanism. For this reason, only a booster shot with tetanus is sufficient if the exposure to the pathogen is within less than 10 years and if the exposure is minimal (wounds are relative superficial). In a situation where pathogen has a short incubation period, the pathogenic mechanism is such that only a small amount of pathogenic molecules could be fatal (*e.g.*, tetanus and diphtheria) and/or bolus of infection is relatively large, both passive and active post exposure immunization are essential. Passive prophylactic immunization is also normal in cases of defects in the immune system, such as hypogammaglobulinemias.

Adverse effects of immunization: Active immunization may cause fever, malaise and discomfort. Some vaccine may also cause joint pains or arthritis (rubella), convulsions, sometimes fatal (pertussis), or neurological disorders (influenza). Allergies to egg may develop as a consequence of viral vaccines produced in egg (measles, mumps, influenza, yellow fever). Booster shots result in more pronounced inflammatory effects than the primary immunization. The noticeable and serious side effects documented have been those following the DTP vaccine (Table 2). Most of these were attributable to the whole pertussis component of the vaccine and have been eliminated since the use of the acellular pertussis preparation.

Table 2. Approximate rates of adverse event occurring within 48 hours DTP vaccination

Event	Frequency
Local:	
redness, swelling, pain	1 in 2-3 doses
Mild/moderate systemic:	
fever, drowsiness, fretfulness	1 in 2-3 doses
vomiting, anorexia	1 in 5-15 doses
More serious systemic:	
persistent crying, fever	1 in 100-300 doses
collapse, convulsions	1 in 1750 doses
acute encephalopathy	1 in 100,000 doses
permanent neurological deficit	1 in 300,000 doses

You have learned:

- Different modes of acquiring immunity
- Which mode is used or applicable in what situation
- Advantages and disadvantages of different modes of immunization
- Rationale for vaccine design
- Adjuvants and their uses
- Risk and benefits of vaccination

Reference:

Exosome Function: From Tumor Immunology to Pathogen Biology by Jeffrey S. Schorey, Sanchita Bhatnagar

Cellular and Molecular Immunology, Updated Edition: With STUDENT CONSULT Online Access, 5e (Cellular and Molecular Immunology, Abbas) 5th Edition by Abul K. Abbas MBBS (Author), Andrew H. H. Lichtman MD PhD (Author)