Tumor Immunology
Cancer cells are different

- Escape normal intercellular communication
- Allow for rapid growth
- Increased mobility of cells
- Invade tissues
- Undergo metastasis
- Evade the immune system
Tumor Immunology is concerned with the study of:

- Antigenic properties of tumor cells.
- Host immune response to tumor cells.
- Immunologic consequences to the host of the growth of tumor cells.
- The means by which the immune system can be modulated to recognize tumor cells and promote tumor eradication.
Introduction 2

- Sensitive immunologic techniques might detect tumor-specific material.

- Studies conducted in the 1950’s and the 1960’s provided the first evidence for the existence of “tumor – specific” rejection antigens.

- In the absence of molecular information, it remained unclear whether tumors indeed expressed truly tumor – specific antigens.
Two recent discoveries

First: T-cells can detect intracellular protein antigens.

Second: truly tumor-specific antigens encoded by tumor-specific somatic mutations exist.
• Tumor-specific diagnostic markers shared by many or all cancers are lacking.

• The only reliable diagnosis of cancer is usually made by the pathologist using histological or cytological techniques.
Early studies suggested that immunization against cancer was possible.

Evidence for tumor-specific rejection of transplanted cancers

- Inbred mice could be immunized against chemically induced cancers.
- Tumor cells did not immunize against normal skin nor did normal tissue immunize against the tumor.

However, at least some of these antigens may be encoded by normal genes that are only expressed at an undetectable level on normal cells.
Early experiments (1950s) showed that mice can be immunized against tumors.
Are antigens unique for an individual cancer or are they shared by other cancers?

- The strongest immunologic protection against challenge with cancer cells was individually tumor-specific.

- Hyperimmunization only led to protective immunity against challenge with other less immunogenic tumors.

- Resistance induced by immunization with unrelated tumors is usually weak, rather short-lived and sensitive to gamma radiation or requires hyperimmunization.
Tumor associated transplantation antigens: shared Ag on virally induced tumors

infect an oncogenic virus (SV40)

2 weeks

remove tumors A & B

isolate tumor cells

immunize with irradiated tumor A cells

challenge with live tumor A

2 weeks

no tumor

challenge with live tumor B

2 weeks

no tumor
Tumor associated transplantation antigens: unique Ag on chemically induced tumors

1. Inject carcinogen (MCA)
2. 2 weeks
3. Remove tumors A & B
4. Isolate tumor cells
5. Immunize with irradiated tumor A cells
6. 2 weeks
7. Challenge with live tumor A
8. 2 weeks
9. No tumor
10. Isolate tumor cells
11. Challenge with live tumor B
12. 2 weeks
13. Tumor
Rejection Antigens on Tumors

- Transplantation Immunity is Primarily T-cell Mediated (CTLs).

- Multiplicity of unique tumor antigens expressed on a single cancer cell.
  - Is the antigenicity of a given tumor due to single or multiple independent components?
  - CTL – defined tumor antigenicity is composed of multiple independent unique epitopes.
  - Whether they reside on one or on different molecules is presently unclear; in any case, these epitopes appear to be functionally independent.
Tumor immunity can be transferred to naive mice by T lymphocytes
Types of Tumor Antigens:

- Tumor specific antigens
  - Mutation, Clonal amplification, Gene activation

- Tumor associated antigens
  - Oncospermatogonal antigens
  - Differentiation antigens
  - Oncofetal and carcinoembryonic antigens
  - Clonal antigens

- Tumor antigens encoded by viral genes
Rejection Antigens on Tumors

Tumor Antigens Encoded by Mutant Cellular Genes (Tumor-specific antigens)

• Three possible mechanisms may lead to the appearance of these antigens: **Mutation, Clonal amplification, or Gene activation.**

• **Clonal amplification:** single cells expressing a particular normal antigen originally present in small numbers not sufficient to be recognized until amplified.

• **Gene Activation:** normal genes that were previously silent may be activated by the carcinogen.
Mutation: unique tumor antigens on tumors induced by carcinogens are products of mutated genes, possibly single genes with “hot spots” for mutations.

These antigens do not seem to involve a single gene family but rather involve multiple different unrelated genes.

Most of these mutations do not appear to be located in random sites, but rather occur in genes that code for functionally important parts of the expressed proteins.
Unique antigens can elicit strong tumor-specific rejection of cancers and may, therefore, be important target antigens for immunotherapy.

Selected mutations occur preferentially in certain genes and often in highly selected locations in these genes.

Many of these mutations are causally related to and specific for the malignant process.
Rejection Antigens on Tumors 8

- **Tumor Antigens Encoded by Normal Cellular Genes (Tumor Associated Antigens)**

- All are encoded by nonmutant cellular genes that are expressed by cancer and some normal adult cells.

- They are not tumor-specific that is why the name.

- The extent and time of their expression during development or differentiation of normal cells and tissues can vary considerably.
Mechanisms for an operational relative tumor specificity for tumor-associated antigens:

- Expression at much higher levels (10-100 fold)

- A better access of the antigen-specific effector cell than to normal cells.

- The expression of the epitope on the normal cells is hidden from the immune system by more complete glycosylation

- Lack of expression of MHC molecules
Oncospermatogonial Antigens

- Certain CTL-recognized antigens on human melanomas and several other cancers (e.g. MAGE 1,2,3) were reported to be encoded by normal genes that were found to be expressed only in the malignant cells.

- Further research revealed that these antigens were also expressed by normal spermatogonia and spermatocytes and possibly other normal cells.
Differentiation Antigens

• Some antigens are expressed on tumor cells as well as on nonmalignant cells of the cell lineage from which the tumor developed (differentiation markers).

• These antigens represent a very diverse group of proteins, glycoproteins, and glycolipids.

• The presence of these normal differentiation antigens can help to determine the organ or cell type of origin.
The use of differentiation markers for histologic or cytologic tumor classification has pitfalls.

- First, cancer cells occasionally express differentiation antigens normally not expressed in the cell lineage from which the tumor originated (aberrant expression).
- Second, differentiation markers can be lost during tumor progression, leaving no clue as to the cancer’s tissue of origin.
Some tissue-specific antigens are detected only cytologically or histologically, whereas others can be used as serum markers (PSA).

There are no immunologic serum markers presently available that are specific for cancer, because their levels are also elevated in a variety of nonmalignant diseases and conditions.
Oncofetal and Carcinoembryonic Antigens

- Expression of antigens that serologically cross react with normal embryonic tissue
- Two different mechanisms:
  - First: an aberrant activation or “derepression” of genes that are supposedly completely silent in adult nonmalignant cells
  - Second: various types of injury or disease.
Some Tumor Associated Antigens

- Human Chorionic Gonadotropin (HCG)
- Alpha Fetoprotein (AFP)
- Prostate Specific Antigen (PSA)
- Mucin CA 125 (glycoprotein molecules on both normal epithelium and carcinomas)
- Carcinoembryonic Antigen (CEA)
Carcinoembryonic Antigen (CEA)

- Discovered as a tumor-specific antigen in human colon carcinoma and as a fetal antigen restricted to fetal gut, pancreas and liver in the first two trimesters of gestation.

- Present in low levels in nonmalignant, nonfetal adult tissue such as normal colonic mucosa, lung and lactating breast tissue.

- Found in other malignancies outside the GI tract (lung and breast) and in the absence of malignancy (smokers and inflammatory bowel disease).
Carcinoembryonic antigen: clinical use

- Adjunct in diagnosis
- Staging and prognosis
- Monitoring response to therapy
- Detection of tumor recurrence
CEA TUMOR ANTIGEN IN COLON CANCER

![Graph showing CEA levels over time following surgical removal of tumor]

- **Normal Range**
- **Surgical Removal of Tumor**
- **Clinical Manifestations Appear**

**Time in Days**

- 0
- 100
- 200
- 300
- 400
Alpha Fetoprotein (AFP)

- Produced by fetal liver and yolk sac cells and present in small amounts in the serum of normal adults.

- Elevated in some patients with cancer of the liver or testis and also in some patients with various nonmalignant liver diseases.

- Assay of AFP can detect primary liver cancer at a time when the cancer is treatable, and AFP assays are also used for monitoring patients after therapy.
Alpha fetoprotein: concentrations

- **Normal concentration:** <20 ng/ml
- **Abnormal concentrations**
  - 100-350 possible hepatoma
  - 350-500 probable hepatoma
  - 500-1000 likely hepatoma
  - >1000 HEPATOMA
Clonal Antigens

- Expressed only on a few normal adult cells (surface Ig in B cell malignancies).

Tumor Antigens Encoded by Viral Genes

- DNA tumor viruses (T antigen in polyoma viruses, E1A/E1B in adeonoviruses and E6/E7 in human papilloma viruses)
- RNA tumor viruses
<table>
<thead>
<tr>
<th>Class of antigen</th>
<th>Antigen</th>
<th>Nature of antigen</th>
<th>Tumor type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-specific mutated oncogene or tumor suppressor</td>
<td>Cyclin-dependent kinase 4</td>
<td>Cell-cycle regulator</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>β-Catenin</td>
<td>Relay in signal transduction pathway</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Caspase-8</td>
<td>Regulator of apoptosis</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Germ cell</td>
<td>MAGE-1</td>
<td>Normal testicular proteins</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>MAGE-3</td>
<td>Normal testicular proteins</td>
<td>Breast Glioma</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Tyrosinase</td>
<td>Enzyme in pathway of melanin synthesis</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Surface Ig</td>
<td>Specific antibody after gene rearrangements in B-cell clone</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Abnormal gene expression</td>
<td>HER-2/neu</td>
<td>Receptor tyrosine kinase</td>
<td>Breast Ovary</td>
</tr>
<tr>
<td>Abnormal post-translational modification</td>
<td>MUC-1</td>
<td>Underglycosylated mucin</td>
<td>Breast Pancreas</td>
</tr>
<tr>
<td>Oncoviral protein</td>
<td>HPV type 16, E6 and E7 proteins</td>
<td>Viral transforming gene products</td>
<td>Cervical carcinoma</td>
</tr>
</tbody>
</table>

Figure 14-11 Immunobiology, 6/e. (© Garland Science 2005)
Immunologic Factors Influencing the incidence of cancer

Immunosurveillance of Tumor Development

- Cancer would occur at an “incredible frequency” if host defense did not prevent the outgrowth of cancer cells that arise continuously.

- The primary reason for development of T-cell-mediated immunity during the evolution of vertebrates was for specific defense against altered self or neoplastic cells.

- The term immunosurveillance was coined to describe the concept of a natural immunologic host resistance against the development of cancer.
Tumor Surveillance Hypothesis

• formally proposed by Thomas and Burnet in 1957.

• States that the immune system actively protects the host against altered self-cells including those that have undergone transformation.

• **Mechanisms include:**
  - Macrophage/Dendritic cell attack or antigen presentation
  - CD8 cell-mediated cytotoxicity
  - Antibody dependent cell mediated cytotoxicity (ADCC) by Natural killer cells
1) The presence of tumor cells and tumor antigens initiates the release of “danger” cytokines such as IFNα and heat shock proteins (HSP). These cause the activation and maturation of dendritic cells such that they present tumor antigens to CD8 and CD4 cells. Subsequent T cytotoxic destruction of the tumor cells occurs.
Immunologic Factors Influencing the incidence of cancer

• Immunosurveillance is effective against virally induced cancers, but not against most forms of cancer induced by chemical or physical carcinogens, with the possible exception of UV-induced tumors.

Stimulation of Tumor Development

• Weak immune responses stimulate tumor growth whereas strong ones suppress it.
• A possible role for inflammatory infiltrate and cytokines?
Effector Mechanisms in cancer immunity

- T-lymphocytes (mainly CD8^+ T cells)

- NK and LAK cells.

- Antibodies (poorly understood).

- Macrophages and Neutrophils (indirectly)

- Cytokines.
Tumor antigen or tumor cell

APC

MHC I

T cytotoxic cell

T cytotoxic memory cells

T cytotoxic effector cells

IL-1

MHC II

T helper cell 1

Interferon

IL-2

T helper Memory cell

T helper Effector cell

T helper 2 cell

B Cell

Eosinophil

Perforins, apoptotic signals

Endogenous antigen

Cancer Cell

Summary

Generally ineffective tumor surveillance, but some ADCC
Factors Limiting Effective Tumor Immunity

- Tumor cells can escape or the host fail to elicit tumor-specific immune responses by various mechanisms.

- Cancer cells are genotypically and phenotypically less stable than normal cells and can rapidly change antigenicity to escape immune destruction.
Mechanisms of Immune Escape

Tumor-related:

- Failure of the tumor to provide a suitable target (defective immunosensitivity)
  - Lack of antigenic epitope
  - Lack of MHC class I molecule
  - Deficient antigen processing by tumor cell
  - Antigenic modulation
  - Antigenic masking of the tumor
  - Resistance of tumor cell to tumoricidal effector pathway
Mechanisms of Immune Escape 2

- Failure of the tumor to induce an effective immune response (defective immunogenicity)
  - Lack of antigenic epitope
  - Decreased MHC or antigen expression by the tumor
  - Lack of a co-stimulatory signal
  - Production of inhibitory substances by the tumor
  - Shedding of antigen and tolerance induction
  - Induction of apoptosis in T cells by expression of Fas ligand by cancer cells
  - Induction of T cell signaling defects by tumor burden
Host-related

- Failure of the host to respond to an antigenic tumor
  - Immune suppression or deficiency of host
  - Deficient presentation of tumor antigens by host antigen-presenting cells
  - Failure of host effectors to reach the tumor

- Failure of host to kill variant tumor cells because of immunodominant antigens on parental tumor cells
Negative regulation of tumor immunosurveillance

T regulatory cell inhibits CD4 and CD8 cells by direct contact and secretion of TGF-β

NK cell inhibits CD8 cell activation through several steps of IL-13 secretion
Escape from immunosurveillance

Lack of Neo-antigens
Escape from immunosurveillance

Lack of co-stimulatory molecules
Escape from immunosurveillance

Lack of class I MHC
Escape from immunosurveillance

Tumors secrete
Immunosuppressive molecules
Tumor cells induce apoptosis in T lymphocytes via FAS activation

1) Cancer cells express FAS ligand
2) Bind to FAS receptor on T lymphocytes leading to apoptosis
Escape from immunosurveillance

Tumors shed their neo-antigens
Immunotherapy of cancer

- **Multiple immunotherapeutic strategies**
  - **Non specific**
    - BCG, *Corynebacterium parvum*
    - Cytokines (IL-2, IFN-γ, IL-12, IL-6, G-CSF, TNF)
  - **Specific**
    - Antibodies and toxins or drugs.
    - Adoptive transfer of effectors cells (lymphocytes) with or without IL-2 treatment.
    - Active immunization or tumor vaccines (still experimental).
# Systemic cytokine therapy for tumors

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Tumor rejection in animals</th>
<th>Clinical trials</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-2</td>
<td>Yes</td>
<td>Melanoma, renal cancer, colon cancer; limited success (&lt;15% response rate)</td>
<td>Vascular leak, shock, pulmonary edema</td>
</tr>
<tr>
<td>Interferon-α</td>
<td>No</td>
<td>Approved for melanoma</td>
<td>Fever, fatigue</td>
</tr>
<tr>
<td>TNF</td>
<td>Only with local administration</td>
<td>Sarcoma, melanoma (isolated limb perfusion)</td>
<td>Septic shock syndrome</td>
</tr>
<tr>
<td>Interleukin-12</td>
<td>Variable</td>
<td>Toxicity trials (phase I) in melanoma, others</td>
<td>Abnormal liver function</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>No</td>
<td>In routine use to promote bone marrow recovery</td>
<td>Bone pain</td>
</tr>
</tbody>
</table>

*Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor.*
## Immunotherapy with cytokine gene-transfected tumor cells

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Tumor rejection in animals</th>
<th>Inflammatory infiltrate</th>
<th>Immunity against parental tumor (animal models)</th>
<th>Clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-2</td>
<td>Yes; mediated by T cells</td>
<td>Lymphocytes, neutrophils</td>
<td>In some cases of renal cancer, melanoma</td>
<td>Renal cancer, melanoma</td>
</tr>
<tr>
<td>Interleukin-4</td>
<td>Yes</td>
<td>Eosinophils, macrophages</td>
<td>No long-lasting immunity in human trials</td>
<td>Melanoma, renal cancer</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>Variable</td>
<td>Macrophages, other cells</td>
<td>Sometimes</td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>Variable</td>
<td>Neutrophils and lymphocytes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Yes</td>
<td>Macrophages, other cells</td>
<td>Yes (long-lived T cell immunity)</td>
<td>Renal cancer</td>
</tr>
<tr>
<td>Interleukin-3</td>
<td>Sometimes</td>
<td>Macrophages, other cells</td>
<td>Sometimes</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor.*
## Approved Anti-tumor mAb

<table>
<thead>
<tr>
<th>Specificity of antibody</th>
<th>Form of antibody used</th>
<th>Clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Her-2/Neu</td>
<td>Humanized mouse monoclonal</td>
<td>Breast cancer (approved for clinical use)</td>
</tr>
<tr>
<td>CD20 (B cell marker)</td>
<td>Humanized mouse monoclonal</td>
<td>B cell lymphoma</td>
</tr>
<tr>
<td>CD10</td>
<td>Humanized mouse monoclonal, immunotoxin</td>
<td>B cell lymphoma; in routine use to purge bone marrow of residual tumor cells</td>
</tr>
<tr>
<td>CEA</td>
<td>Humanized mouse monoclonal</td>
<td>Gastrointestinal cancers, lung cancer</td>
</tr>
<tr>
<td>CA-125</td>
<td>Mouse monoclonal</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td>GD3 ganglioside</td>
<td>Humanized mouse monoclonal</td>
<td>Melanoma</td>
</tr>
</tbody>
</table>

Abbreviation: CEA, carcinoembryonic antigen.
Enhancing antibody cytotoxicity for cancer therapy

Diagram showing the conjugation of drugs and toxins to antibodies for cancer therapy.

Chemical conjugation e.g., ricin

Dissociate cell-binding subunit

Toxin

Recombinant fusion protein e.g., scFv - toxin

Chemical conjugation e.g., ricin

Dissociate cell-binding subunit

Toxin

Recombinant fusion protein e.g., scFv - toxin
### Types of Tumor Vaccines

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Vaccine preparation</th>
<th>Animal models</th>
<th>Clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Killed tumor vaccine</strong></td>
<td>Killed tumor cells + adjuvants</td>
<td>Melanoma, colon cancer, others</td>
<td>Melanoma, colon cancer</td>
</tr>
<tr>
<td></td>
<td>Tumor cell lysates + adjuvants</td>
<td>Sarcoma</td>
<td>Melanoma</td>
</tr>
<tr>
<td><strong>Purified tumor antigens</strong></td>
<td>Melanoma antigens</td>
<td>Melanoma</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Heat shock proteins</td>
<td>Various</td>
<td>Melanoma, renal cancer, sarcoma</td>
</tr>
<tr>
<td><strong>Professional APC-based vaccines</strong></td>
<td>Dendritic cells pulsed with tumor antigens</td>
<td>Melanoma, B cell lymphoma, sarcoma</td>
<td>Melanoma, non-Hodgkin's lymphoma, prostate cancer, others</td>
</tr>
<tr>
<td></td>
<td>Dendritic cells transfected with genes encoding tumor antigens</td>
<td>Melanoma, colon cancer</td>
<td>Various carcinomas</td>
</tr>
<tr>
<td><strong>Cytokine- and costimulator-enhanced vaccines</strong></td>
<td>Tumor cells transfected with cytokine or B7 genes</td>
<td>Renal cancer, sarcoma, B cell leukemia, lung cancer</td>
<td>Melanoma, sarcoma, others</td>
</tr>
<tr>
<td></td>
<td>APCs transfected with cytokine genes and pulsed with tumor antigens</td>
<td></td>
<td>Melanoma, renal cancer, others</td>
</tr>
<tr>
<td><strong>DNA vaccines</strong></td>
<td>Immunization with plasmids encoding tumor antigens</td>
<td>Melanoma</td>
<td>Melanoma</td>
</tr>
<tr>
<td><strong>Viral vectors</strong></td>
<td>Adenovirus, vaccinia virus encoding tumor antigen + cytokines</td>
<td>Melanoma, sarcoma</td>
<td>Melanoma</td>
</tr>
</tbody>
</table>

**Abbreviations:** APC, antigen-presenting cell.
Vaccine to elicit T cell response

Induction of anti-tumor T cell response (cross-priming)

Effector phase of anti-tumor CTL response

Tumor cells and antigens ingested by host APCs

Professional APC

Phagocytosed tumor cell

Costimulator

CD8+ T cell

Differentiation of tumor-specific T cells

Tumor-specific CD8+ CTL recognizes tumor cell

Killing of tumor cell

CD4+ helper T lymphocyte

Cytokines

Copyright © 2004, 2001 Elsevier Inc. All rights reserved.
Tumor vaccines - Targeting DCs

A

Vaccinate with tumor-antigen pulsed dendritic cell

Dendritic cells pulsed with tumor antigens

CD8+ T cell

Activation of tumor-specific T cells

B

Plasmid expressing cDNA encoding tumor antigen

Vaccinate with DNA or transfected dendritic cell

Dendritic cells transfected with plasmid expressing tumor antigen

APC producing tumor antigen

CD8+ T cell

Activation of tumor-specific T cells

© Elsevier, Abbas et al; Cellular and Molecular Immunology 6e - www.studentconsult.com
Enhancement of tumor immunogenicity

**A**
Vaccinate with tumor cell expressing costimulators or IL-2
- Tumor cell transfected with gene for lymphocyte costimulator (e.g., B7) or IL-2
- CD8+ T cell
- B7-expressing tumor cell stimulates tumor-specific T cell
- IL-2 enhances proliferation and differentiation of tumor-specific T cells
- Activation of tumor-specific T cells

**B**
Vaccinate with tumor cell expressing GM-CSF
- Tumor cell transfected with gene for GM-CSF
- GM-CSF promotes recruitment and maturation of dendritic cells
- APC
- Dendritic cell ingests, processes, and presents tumor antigens to tumor-specific T cells
- GM-CSF
- Phagocytosed tumor cell
- CD8+ T cell
- Activation of tumor-specific T cells