

TUMOR IMMUNOLOGY

- **Tumor antigens**
- **Effector mechanisms in anti-tumor immunity**
- **Mechanisms of tumor evasion of the immune system**
- **Immunotherapy for tumors**

Immunosurveillance

- An hypothesis that states that *a physiologic function of the immune system is to recognize and destroy malignantly transformed cells before they grow into tumors.*
- Proposed by Paul Ehrlich, Macfarlane Burnet and Lewis Thomas
- Implies that cells of the immune system recognize something “foreign” on transformed/tumor cells.

Evidence in Support of Immunosurveillance (I)

- Immunodeficient individuals are more likely to develop certain types of tumors than immunocompetent individuals.

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- Immunodeficient individuals are more likely to develop certain types of tumors than immunocompetent individuals.
- Clinicopathologic correlations suggest that lymphocytic infiltrates in some tumors (e.g. medullary breast carcinoma, malignant melanoma) are associated with a better prognosis compared to histologically similar tumors without infiltrates

Evidence in Support of Immunosurveillance (II)

- Histologic evidence indicates that active immune responses occur within tumors or in draining lymph nodes.

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- There is ample evidence that T and B lymphocytes specific for tumor surface molecules have been activated and expanded in tumor patients.

Tumor Immunosurveillance; Qualified

- Since common cancers (e.g. carcinomas of lung, colon, breast, prostate) arise frequently in immunocompetent individuals, immunosurveillance is often *not* effective.

Tumor Immunosurveillance; Qualified

See Immunobiology, by Janeway, C., Travers, P., Walport, M. and Capra, J., Garland Publishing, 5th edition, 2001 & Cellular and Molecular Immunology by Abbas, A., Pober, J., and Lichtman, A., W B Saunders; 4th edition.

Transplantation Antigens on Chemically Induced Tumors

Immunization with killed tumor cells from:	Challenge with live tumor cells from:	Result	Conclusion
Chemically induced sarcoma A	Chemically induced sarcoma A	No growth	Immunity is specific for individual tumor
Chemically induced sarcoma A	Chemically induced sarcoma B	Growth of tumor B	

Transplantation Antigens on Virally Induced Tumors

Immunization with killed tumor cells from:	Challenge with live tumor cells from:	Result	Conclusion
MSV -induced sarcoma A	MSV-induced sarcoma A	No growth	Immunity to virus induced tumors is virus-specific
MSV-induced sarcoma A	MSV-induced sarcoma B	No growth	
MSV-induced sarcoma A	Chemically - induced sarcoma C	Growth	
MSV -induced sarcoma A	MuLV-induced sarcoma D	Growth	

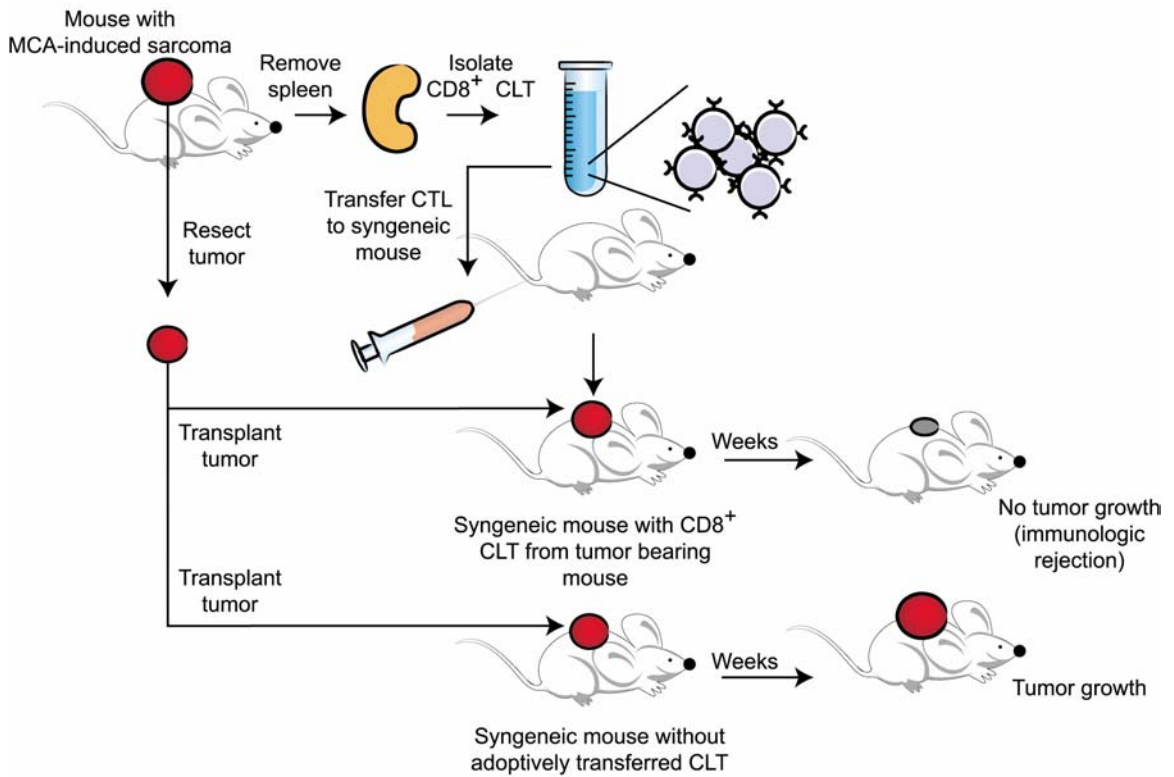
Patterns of Tumor Antigen Expression

- Tumor specific antigens (TSAs): Expressed on tumor cells but not normal cells.
 - Unique tumor antigens on one tumor only
 - Antigens shared between tumors of same type
 - Tumor-specific transplantation antigens (TSTAs)
- Tumor associated antigens (TAAs): Expressed on normal cells and tumor cells.
 - Differentiation antigens

TUMOR ANTIGENS

- Tumor Antigens Recognized by Host T Lymphocytes
- Tumor Antigens Recognized by Antibodies
 - Antibodies produced by host humoral responses
 - Antibodies raised in animals used as diagnostic, therapeutic agents

IMMUNITY TO TRANSPLANTED TUMORS CAN BE TRANSFERRED BY CTLs



IDENTIFICATION OF TUMOR ANTIGENS RECOGNIZED BY T LYMPHOCYTES

- Transplantation studies of tumors in rodents.

IDENTIFICATION OF TUMOR ANTIGENS RECOGNIZED BY T LYMPHOCYTES

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Examples of Tumor Antigens that Stimulate T Cell Responses (I)

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Examples of Tumor Antigens that Stimulate T Cell Responses (II)

- Viral gene products in virus-associated malignancies.
 - SV40 T antigen (SV40-induced rat tumors)
 - Human papillomavirus E6 and E7 gene products (human cervical carcinoma)
 - Epstein-Barr virus EBNA-1 gene product (Burkitt's, lymphoma and nasopharyngeal carcinoma)

Melanoma Antigens

- Properties of melanomas that facilitate the study of tumor antigens.
 - Readily excisable from skin
 - Can be grown in tissue culture
 - Often elicit a marked lymphocytic response
- Studies of melanoma antigens
 - Establishment of cloned CTL lines specific for melanomas
 - Identification of melanoma proteins recognized by CTL

Melanoma Tumor Antigens Recognized by CTLs

- Tumor-associated testis-specific antigens- normally not expressed (“silent”) in most tissues
 - MAGE, BAGE, GAGE (expressed by many human melanomas, many types of carcinomas, normal testis)
- Tissue specific antigens:
 - Tyrosinase, Mart-1, gp100 (expressed by normal melanocytes):
- Mutated or aberrantly expressed molecules
 - MUM-1 (point mutation of gene of unknown function)
 - beta-catenin

Tumor Antigens Recognized by T Lymphocytes: Review

- Products of mutated normal cellular genes not related to oncogenesis

Tumor Antigens Recognized by T Lymphocytes

- Products of mutated normal cellular genes not related to oncogenesis
- Products of oncogenes and mutated tumor suppressor genes

Tumor Antigens Recognized by T Lymphocytes

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- Products of normally silent genes

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- Products of oncogenes and mutated tumor suppressor genes
- Products of normally silent genes
- Tumor antigens encoded by genomes of oncogenic viruses
- Tissue-specific differentiation antigens recognized

Tumor-Specific CTLs in Paraneoplastic Cerebellar Degeneration

- Some breast and ovarian cancer patients have paraneoplastic cerebellar degeneration (PCD)
- In these patients there is a specific immune response to a shared tumor and Purkinje cell antigen, cdr2.
- Expanded populations of MHC class I-restricted cdr2-specific CTLs are found in the blood of PCD patients.
- Cross-presentation of apoptotic tumor cells by dendritic cells activates a potent, cdr2-specific CTL response *in vitro*
- In PCD, peripheral activation of cdr2-specific CTLs is likely to contribute to the subsequent development of the autoimmune neuronal degeneration.

Tumor Antigens Recognized by Antibodies

- **Oncofetal antigens**
- Altered glycolipid and glycoprotein antigens
- Tissue specific differentiation antigens

Oncofetal Antigens

- Molecules normally expressed on developing (fetal) but not adult tissues.
- Expression in adult not strictly limited to tumors; low amounts in normal tissues and increased amounts in inflammatory conditions.
- Do not induce protective immune responses.
- Useful as markers that aid in the diagnosis of tumors.

Oncofetal Antigens: Cacinoembryoinc antigen (CEA, CD66)

- Heavily glycosylated membrane protein; may function as adhesion molecule.
- Highly expressed in developing gut, liver and pancreas (1st two trimesters).
- Expressed at low levels on granulocytes and gut epithelial cells in adult.
- Highly expressed by carcinomas of colon, pancreas, stomach and breast, with associated *elevated serum levels*.
- Serum levels also elevated in setting of inflammatory diseases of liver and colon.

Clinical uses of Cacinoembryoinc antigen (CEA, CD66)

- Serum levels are followed after surgical removal of colorectal carcinomas to detect recurrent disease.
- Radiolabelled anti-CEA (^{99}Tc -anti-CEA) used for immunolocalization of tumors.
- Anti-CEA antibodies used for immunohistopathologic diagnosis of tumors.
- Possible target for immunotherpay.

Oncofetal Antigens: Alpha-fetoprotein (AFP)

- α -globulin glycoprotein secreted by yolk-sac and liver during fetal life; replaced by albumin in adult life.
- Serum levels elevated in patients with hepatocellular carcinoma, germ-cell tumors, and some gastric and pancreatic tumors.
- Elevated levels also seen in non-neoplastic liver disease (e.g. cirrhosis).
- Serum levels followed to assess tumor burden after treatment of liver or germ-cell tumors.
- Immunohistochemical detection of AFP in sections of tumors may aid in pathologic diagnosis of tumor type.

Tumor Antigens Recognized by Antibodies

- Oncofetal antigens
- Altered glycolipid and glycoprotein antigens
- Tissue specific differentiation antigens

Altered Glycolipid and Glycoprotein Antigens

- Require multiple enzymes to catalyze sequential addition of carbohydrate groups to protein or lipid cores.
- Due to abnormal expression of these enzymes, many tumors express high levels and/or abnormal forms of surface glycoproteins or glycolipids. (Gangliosides, blood group antigens, mucins.)
- These abnormal cell surface glyco-molecules may contribute to some aspects of the malignant phenotype.
- Xenogenic antibodies have been raised against many of these molecules.
- This class of TAAs is a preferred target for antibody-based cancer-therapy.

Altered Glycolipid and Glycoprotein Antigens: **Gangliosides**

- Neuraminic acid-containing glycosphingolipids)
- GM2 and GD2 present at very high densities on melanomas compared to normal melanocytes.
- Melanoma patients may make antibody responses to these molecules.
- Targets for vaccine and xenogenic antibody therapy

Altered Glycolipid and Glycoprotein Antigens: **Mucins**

- High molecular weight proteoglycans with numerous O-linked CHO side chains
- Relatively tumor specific epitopes on side chains or abnormally exposed core polypeptide.
- CA-125 on ovarian tumors, MUC-1 on breast carcinomas.
- Targets for vaccine and passive antibody based therapies.

Altered Glycolipid and Glycoprotein Antigens: Blood group antigens

- Carbohydrate epitopes on glycosphingolipids or glycoproteins
- T and sialylated Tn antigens aberrantly expressed in carcinomas
- Used in experimental tumor vaccines

Tumor Antigens Recognized by Antibodies

- Oncofetal antigens
- Altered glycolipid and glycoprotein antigens
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Tissue-Specific Tumor Antigens Used in Clinicopathologic Analysis of Tumors

<u>Tissue of Origin</u>	<u>Tumor</u>	<u>Antigens</u>
B lymphocytes	B cell leukemias and lymphomas	CD10 (CALLA), Immunoglobulin
T lymphocytes	T cell leukemias and lymphomas	Interleukin-2 receptor (α chain), T cell receptor, CD45R, CD4/CD8
Prostate	Prostatic carcinoma	Prostate-specific antigen, Prostatic acid-phosphatase
Neural crest-derived	Melanomas	S-100
Epithelial cells	Carcinomas	Cytokeratins

Mechanisms of Tumor Killing

See Immunobiology, by Janeway, C., Travers, P., Walport, M. and Capra, J., Garland Publishing, 5th edition, 2001 & Cellular and Molecular Immunology by Abbas, A., Pober, J., and Lichtman, A., W B Saunders; 4th edition.

Mechanisms of Tumor Killing

- NK cells
 - Recognize lack of normal self class I MHC on some tumors
 - Kill tumor cells in vitro by a perforin/granzyme granule exocytosis mechanisms similar to CTLs
 - May be defense against tumors which have escaped CTL killing

NK Cells: Review

- MHC-I recognition by NK cells is due to the surface expression of inhibitory receptors that bind MHC-I.
- Human NK cells express two families of MHC-I-binding inhibitory receptors,
 - Killer cell inhibitory receptors (KIR) are type I transmembrane Ig superfamily proteins that bind to classical HLA-A, -B and -C molecules
 - CD94/NKG2A receptors are heterodimeric type II transmembrane proteins with C-type lectin domains that bind to the non-classical HLA-E
 - Engagement of KIR and CD94/NKG2A inhibitory receptors with MHC-I dominantly engages SHP-1 tyrosine phosphatases and arrests activation signals derived from numerous receptors interacting with cell surfaces, such as CD2, CD16, NKR-P1, integrins, and several recently identified receptors.

Anti-tumor activity of NK cells

- **NKG2D is a C-type lectin expressed on NK cells that associates with DAP10**
- **The NKG2D-DPA10 complex is a receptor for the nonclassical MHC class I molecule MICA (MHC class I-related chain A)**
- **NKG2D-DPA10 transduces signals that activate NK cells**
- **MICA is broadly expressed in epithelial tumors suggest that the activating NKG2D-DAP10 complex may be involved in innate immune surveillance against these tumors.**

• Wu et al Science 1999 285:730

NK Cell Killing of Tumor Cells

See Immunobiology, by Janeway, C., Travers, P., Walport, M. and Capra, J., Garland Publishing, 5th edition, 2001 & Cellular and Molecular Immunology by Abbas, A., Lichtman, A., and Pober, J., W B Saunders; 4th edition.

Mechanisms of Tumor Killing

- Antibodies against tumor antigens
 - activity demonstrated mainly *in vitro*
 - most tumor-specific antigens do not elicit antibody responses *in vivo*
- Activated macrophages
 - activity demonstrated mainly *in vitro*
 - tumor cells may be more susceptible to macrophage-mediated killing than normal cells

Mechanisms of Tumor Evasion of the Immune System

- Down-regulation of class I MHC expression
- Resistance to killing by CTLs
- Poor induction of helper T cell responses/poor costimulation of T cells
- Immunosuppressive tumor products (e.g. TGF- β)
- Host tolerance to tumor antigens
- Selection of non-immunogenic clones of tumor cells
- Antigenic modulation
- Tumor growth kinetics
- Masking of antigens by glycocalyx

Loss of Mannose-6 phosphate receptor (CI-MPR) expression by tumors

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Immunotherapy of Tumors

- **Passive tumor immunotherapy**
 - Adoptive cellular immunotherapy
 - Anti-tumor antibodies
 - Cytokines
- **Stimulation of active host immune responses**
 - Non-specific stimulation of immune system
 - Vaccination with tumor cells
 - Vaccination with tumor antigens
 - Augmentation of host immunity with costimulators/cytokines
 - Stem cell transplant with graft vs. tumor response

Passive Tumor Immunotherapy

- **Adoptive cellular therapy**
- **Therapy with anti-tumor antibodies**
- **Cytokines**

Adoptive Cellular Immunotherapy

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Adoptive Cellular Immunotherapy

- Intravenous injection of LAK cells or TILs into tumor patients.
- Supplemented with administration of IL-2 and/or chemotherapeutic agents.
- TILs may be transfected with cytokine genes
- Trials with advanced metastatic disease (melanomas) have had variable results.

Passive Tumor Immunotherapy

- Adoptive cellular therapy
- Therapy with anti-tumor antibodies
- Cytokines

Anti-tumor Monoclonal Antibodies

- Antibodies specific for tumor specific or tumor associated cell surface antigens may serve as “magic bullets” that target cancer cells for destruction while sparing most normal tissues
- Mechanisms
 - Fc receptor mediated engagement of phagocytes
 - Complement mediated injury or clearance
 - Target toxins or drugs linked to antibodies
 - Inhibitory signals induced by antibody

Examples of Anti-Tumor mAbs in Clinical Use/Trials

- IL-2 receptor on T cell lymphomas
- sIg idiotypes on B cell lymphomas
- CD20 on B cell lymphomas
- CD33 on myelogenous leukemia cells
- HER2/neu on breast carcinomas
 - **transmembrane tyrosine kinase receptor over-expressed in many carcinomas**

Drug/Toxin/Radioisotope Conjugates of mAbs for Cancer Treatment

- Anti-CD20-radionucleotide conjugates for B cell lymphomas
- Anti-CD33-calicheamicin conjugates for acute myelogenous leukemia.
- Conjugates of anti-tumor mAbs with ricin, diphtheria toxins in

Passive Tumor Immunotherapy

- Adoptive cellular therapy
- Therapy with anti-tumor antibodies
- Cytokines

Cytokine Immunotherapy

- Tumor necrosis factor (TNF)
 - Potent inflammatory mediator; highly toxic
 - Investigational use in isolated limb perfusion Rx of sarcomas
- Type I interferons (IFN- α/β)
- Immunoregulatory and anti-proliferative effects
 - Approved for chronic myeloid and hairy cell leukemias, AIDS-related Kaposi's sarcoma

Cytokine Immunotherapy

- Interleukin-2
 - Immunoregulatory (T cell growth, NK cell activation); high dose Rx highly toxic (vascular leak)
 - Investigational use alone or with adoptive cellular Rx for renal cell carcinoma and melanoma
- Interleukin 12
 - Immunoregulatory (endogenous adjuvant, NK-cell activation)
 - Investigational use

Tumor Immunotherapy:
Stimulation of Active
Host Immune Responses to Tumors

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Modification of Mouse Tumors by Transfected Cytokine Genes

Cytokine	Enhanced Rejection of Transfected Tumor	Inflammatory Infiltrate	Distant Immunity Against Parental Tumor
IL-2	Yes (CD8 ⁺ T cell dependent)	Lymphocytes	Sometimes
IL-4	Yes	Macrophages Eosinophils	Sometimes (CD4 ⁺ and CD8 ⁺ T cell dependent)
GM-CSF	Yes	Immature mononuclear cells	Yes (long lived, CD4 ⁺ and CD8 ⁺ T cell dependent)

Anti-Tumor Vaccines

- Goal: To boost weak cell-mediated immune responses to tumor antigens
 - Both helper T cell and CTL responses
- Form of vaccine
 - Peptides
 - Dendritic cells pulsed with tumor peptides
 - Cells expressing recombinant genes encoding tumor antigens
 - DNA encoding tumor antigens

Bone Marrow Transplant and
Graft vs. Tumor Response

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Bone Marrow Transplant and Graft vs. Tumor Response

- Engraftment of allogeneic bone marrow cells can be accomplished following non-myeloablative conditioning while possibly controlling graft-versus-host disease.
- GVL and GVT effects may be accomplished after non-myeloablative stem cell transplantation (NST) by donor lymphocyte infusion (DLI)

The New England Journal of Medicine

(N Engl J Med 2000; 343:750-8)

Regression of Metastatic Renal-Cell Carcinoma after Nonmyeloablative Allogeneic Peripheral-Blood Stem-Cell Transplantation

Richard Childs, Allen Chernoff, Nathalie Contentin, Erkut Bahceci, David Schrupp, Susan Leitman, Elizabeth J. Read, John Tisdale, Cynthia Dunbar, W. Marston Linehan, Neal S. Young, A. John Barrett, Emmanuel Clave, Diane Epperson, Virginia Mayo

In 10 patients (53 percent) metastatic disease regressed; 3 had a complete response, and 7 had a partial response. The patients who had a complete response remained in remission 27, 25, and 16 months after transplantation. Regression of metastases was delayed, occurring a median of 129 days after transplantation, and often followed the withdrawal of cyclosporine and the establishment of complete donor-T-cell chimerism. These results are consistent with a graft-versus-tumor effect.