Tumor Immunology and Immunotherapy

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- "The Biology of Cancer", Chapter 15, R.A. Weinberg, Garland Science publisher, 2007
- http://www.cancer.gov/cancertopics/treatment/types-of-treatment

Major Questions in Tumor Immunology:

- What cellular or molecular mechanisms, if any, enable the immune system to recognize and attack cancer cells?
- Do these immune mechanisms act as defense to prevent the appearance of tumors?
- Can the immune system be manipulated to attack tumors once they have already formed?

Specific Topics for Today

- Immune responses in normal tissues
- Recognition of cancer cells by the immune system
- Evasion of the immune system by tumor cells
- Passive immunization and cancer vaccines as cancer therapies
- Other anti-cancer immunotherapies

Primary Functions of the Immune System

- Attack foreign infectious agents (viruses, bacteria, parasites, etc.)
- Attack the body's own cells if cells are infected with foreign agents

Types of Immune Responses

Classified based on how the immune response is generated:

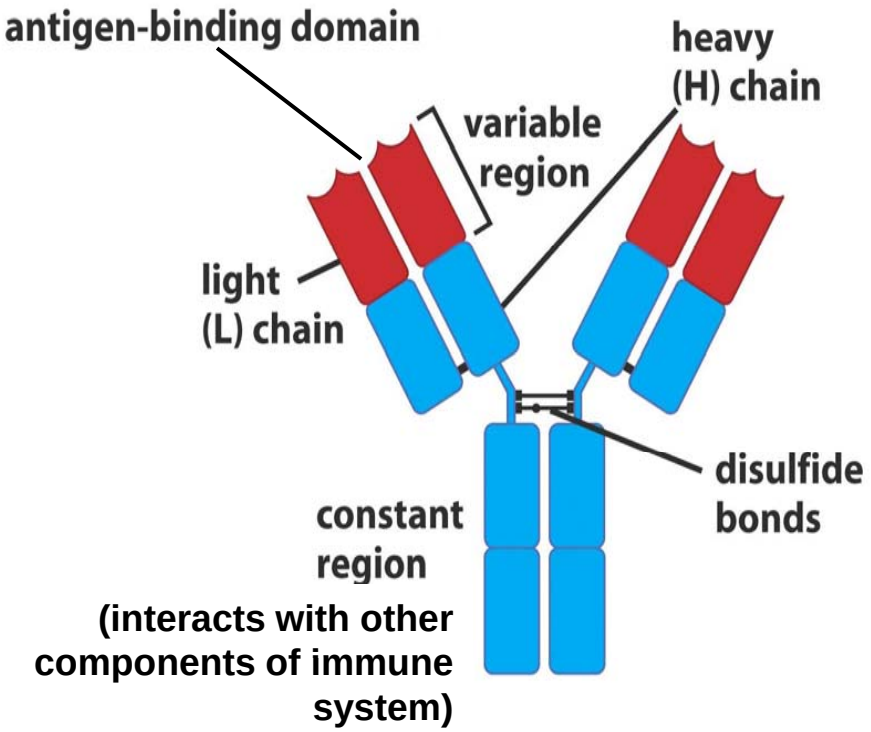
- *Adaptive immune response*: acquired following exposure of an organism to an antigen*
  - Generation of antibodies and cytotoxic T cells
  - Advantage: fast (once established), robust, specific
- *Innate immune response*: response that occurs in absence of prior exposure to antigen
  - NK cells, macrophages and others
  - Advantage: does not require prior exposure

**Antigen*: foreign (usually) molecule (or part of a molecule) recognized by immune system

The Adaptive Immune Response: I.Antigen-specific Antibodies

Antibodies

- Antibodies are proteins produced by B-cell (B-lymphocyte) lineage
- Each B-cell produces antibodies that bind to a single specific target (*i.e.*, antigen)
- Different B-cells target different antigens
- Extremely high diversity of antibodies due to programmed gene rearrangements at multiple stages of B-cell development



Antibody structure (secreted version of the B-cell receptor)

Mechanisms of Antibody Attack

- Antibody (Ab) could bind directly to antigen to neutralize it (*e.g.*, infection blocked if Ab bound to capsid; or GFR signaling blocked by Ab)
- Ab bound to target cell surface could act as "flag" to recruit other immune cells or molecules → eliminate target cells

Antibody-Mediated Elimination of Target Cells by Natural Killer (NK) Cells

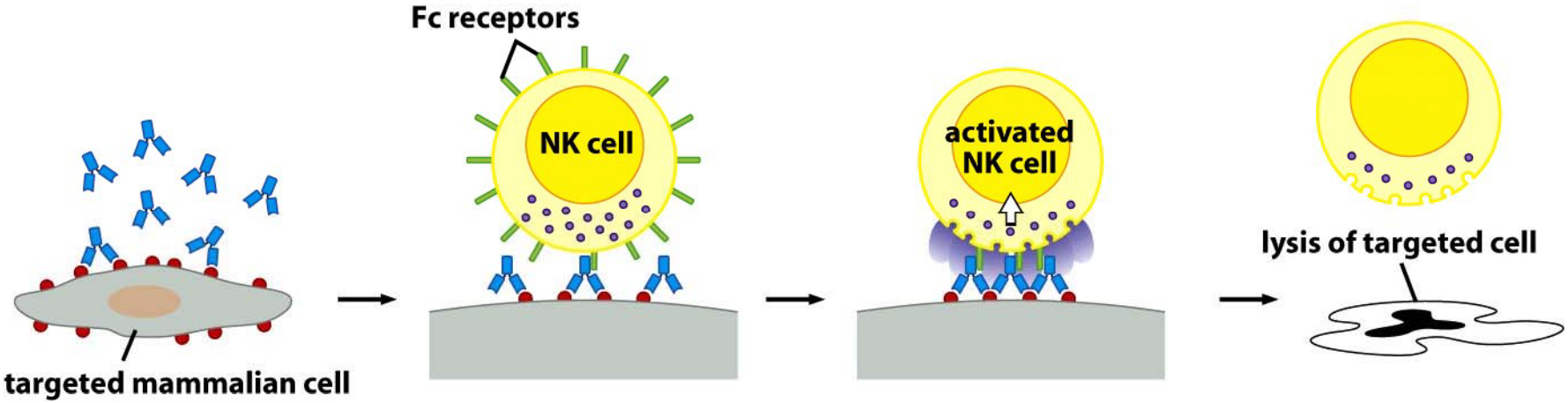
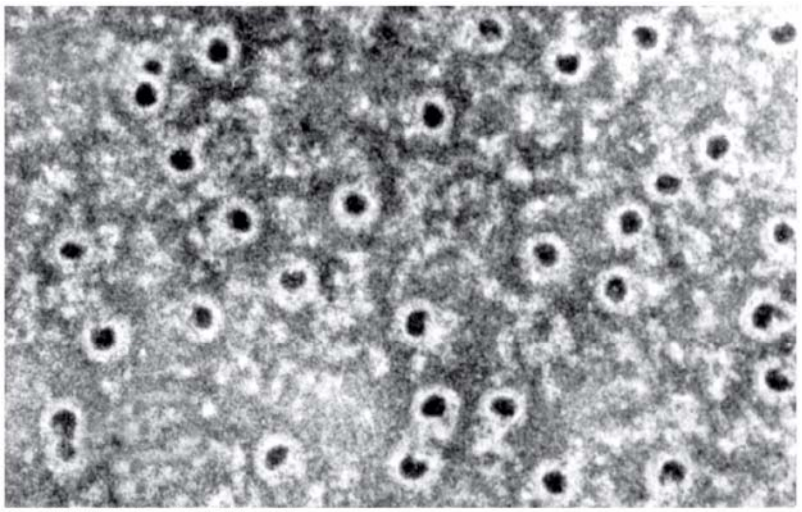


Figure 15-3b The Biology of Cancer (© Garland Science 2007)

Blue: antibodies, red: antigens

Antibody-Mediated Elimination of Target Cells by Complement



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EM of cell perforated by complement

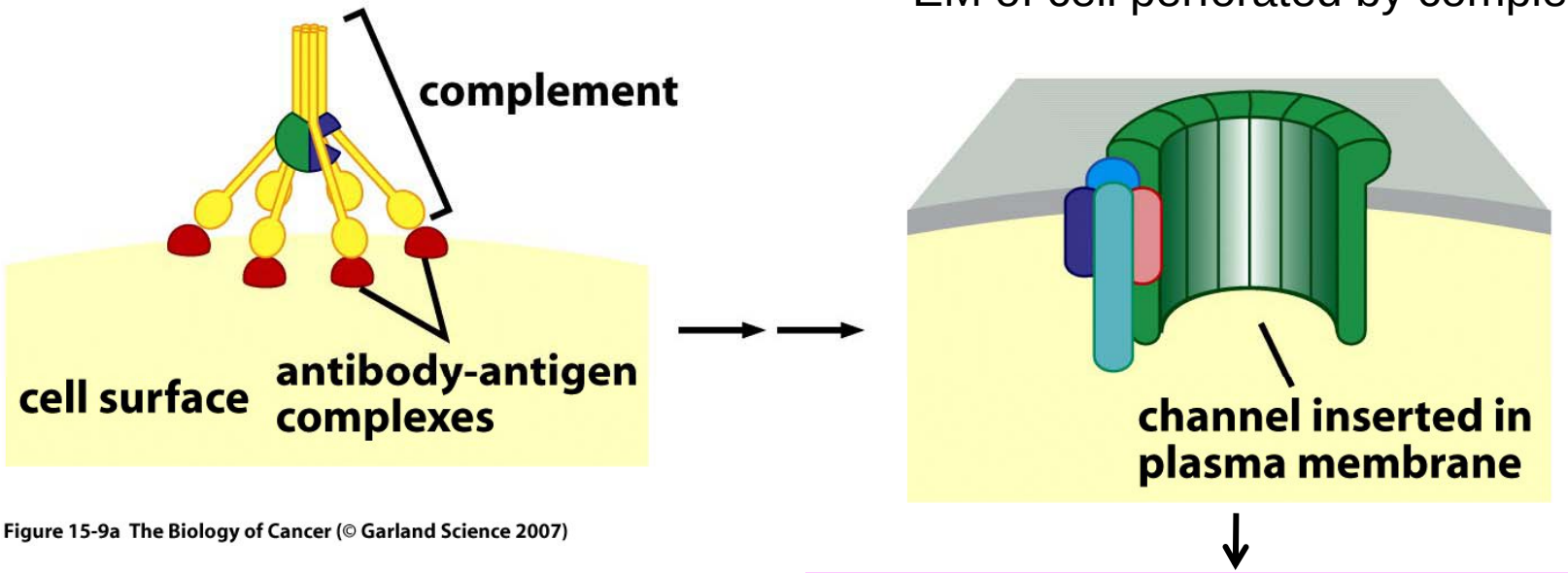


Figure 15-9a The Biology of Cancer (© Garland Science 2007)

- Loss of membrane integrity
- Disruption of proton gradient

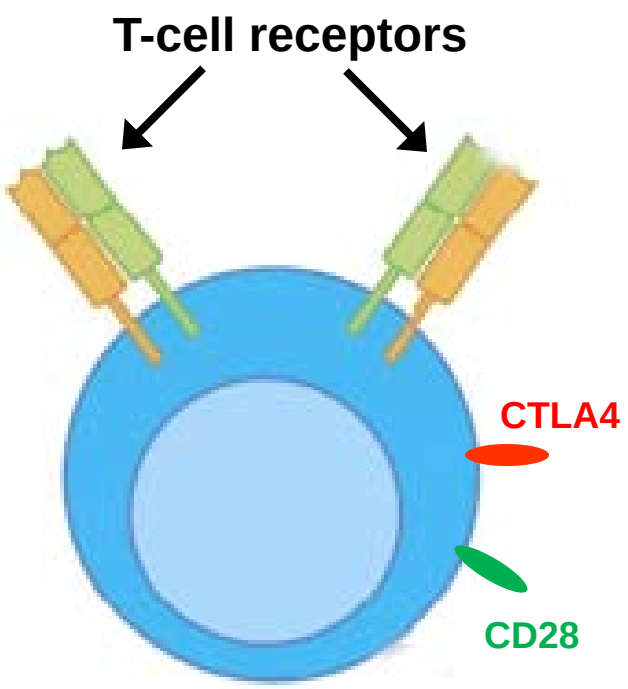
Why does an organism need more than just antibodies for adaptive defense?

- Antibodies cannot recognize antigens unless antigens are extracellular or on the cell surface (Abs can’t cross cell membrane!)
- So, some virus-infected and cancer cells may not be recognized

The Adaptive Immune Response:
II. Target Cell Recognition by Antigen-specific T Cells

T Cells in the Adaptive Response

- Each T cell specific for single antigen (determined by structure of T cell receptor (TCR))
- Populations of T cells have highly diverse TCRs
- Diversity in TCRs due to gene rearrangements during T cell development



T-cell

Subtypes of T Cells

- Helper T cells (T_H): help to activate B cells and T_c cells that are specific for the same antigen as the T_H
- Cytotoxic T lymphocytes (CTL = T_c): kill "foreign" cells
- Regulatory T cells (T_{reg}): counteract T_c and T_H cells when T_{reg} and target T cell are specific for the same antigen (prevent auto-immunity)

➢ Not confined to attacking proteins normally on cell surface (intracellular proteins can also be targets)

Antigen Processing (MHC I Display)

- Some of each protein synthesized within every cell is diverted to specialized proteasomes where it is cleaved to small peptides
- Processed peptides then complexed to MHC (major histocompatibility) class I molecule
- Peptide:MHC complex transported to surface

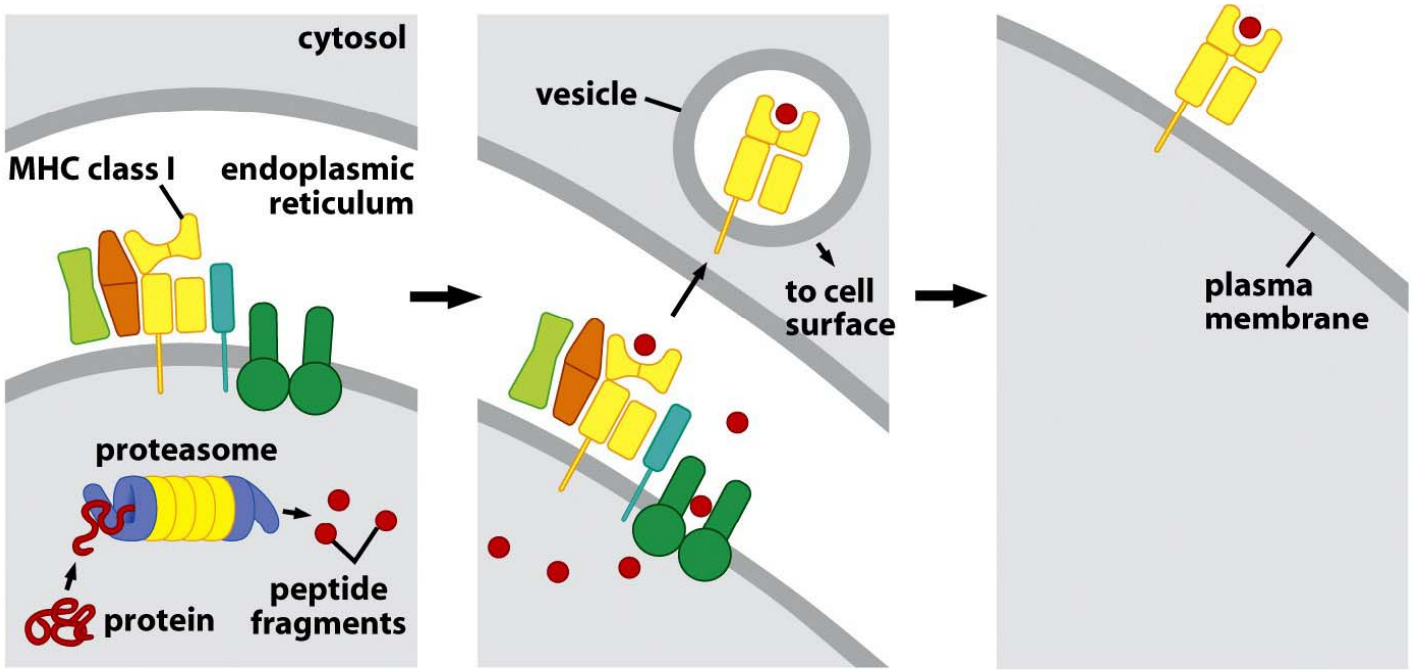


Figure 15-10a The Biology of Cancer (© Garland Science 2007)

Many Oligopeptides are Displayed by Each Cell

- Peptides displayed from:
  - normal cellular proteins
  - viral proteins or
  - mutated proteins
- Continuous process whether or not cells are infected
- Processed peptide-MHC complex recognized by TCR

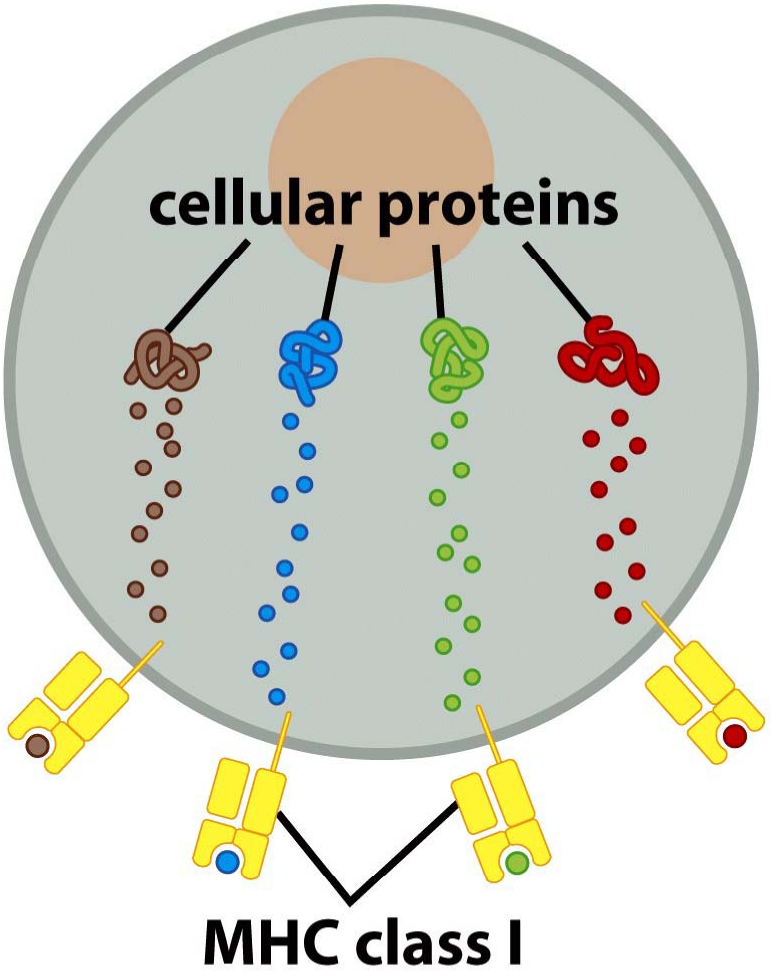


Figure 15-10b The Biology of Cancer (© Garland Science 2007)

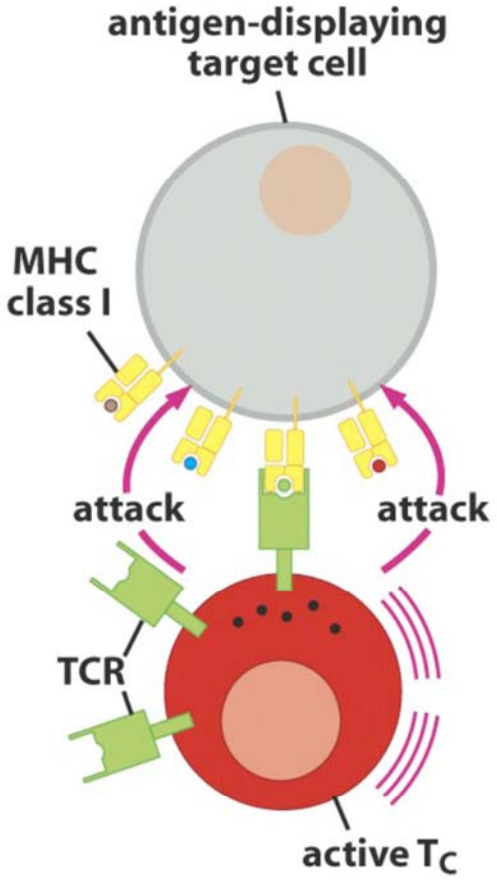
Self vs. Non-self (i.e., Foreign)

- Immune cells that recognize self proteins are dangerous!
  - Cause auto-immunity (rheumatoid arthritis, colitis, lupus erythromatosis)
- Must be eliminated (during development of immune system) or suppressed (suppression mediated by T-regulatory cells)

➢ **Immune tolerance**

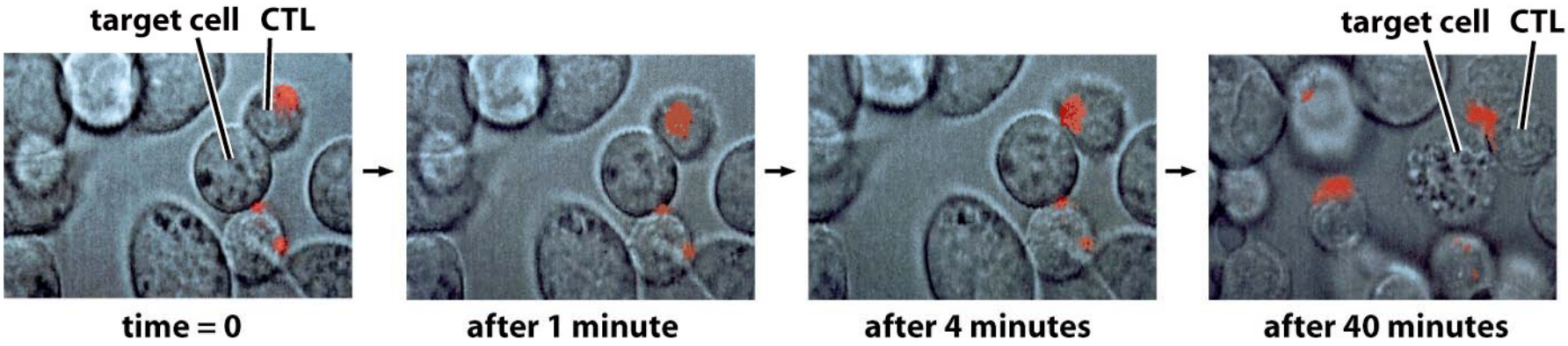
Killing by Cytotoxic T Lymphocyte (T_c)

- Activated CTL (= T_c) recognizes its specific peptide-MHC complex on the target cell
- The T_c kills the target cell by one of two mechanisms:
  - Releasing toxic proteins into the target cells
  - Activation of the Fas Death receptor on the target cell



Destruction of Target Cell by Cytotoxic Granule Release

- TCR binds CTL to its target
- Intracellular cytotoxic granules containing perforin and granzyme B (red) migrate toward synapse with target cell
- Granules released, granzyme B cleaves caspases in target cell, inducing apoptosis



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Fas Death Receptor Activation

FasL on T_c binds Fas on target cell and activates the death receptor apoptotic pathway

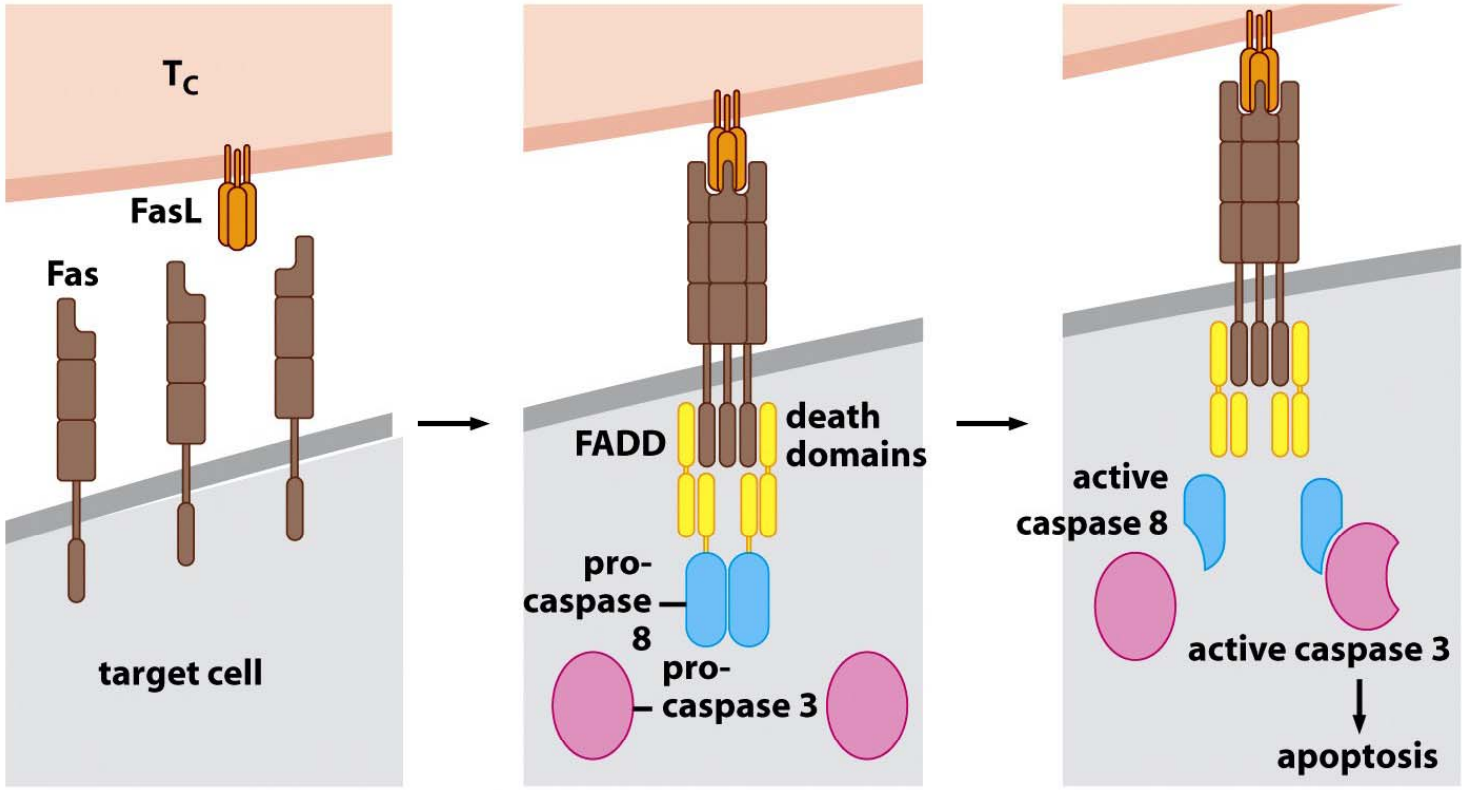


Figure 15-12c The Biology of Cancer (© Garland Science 2007)

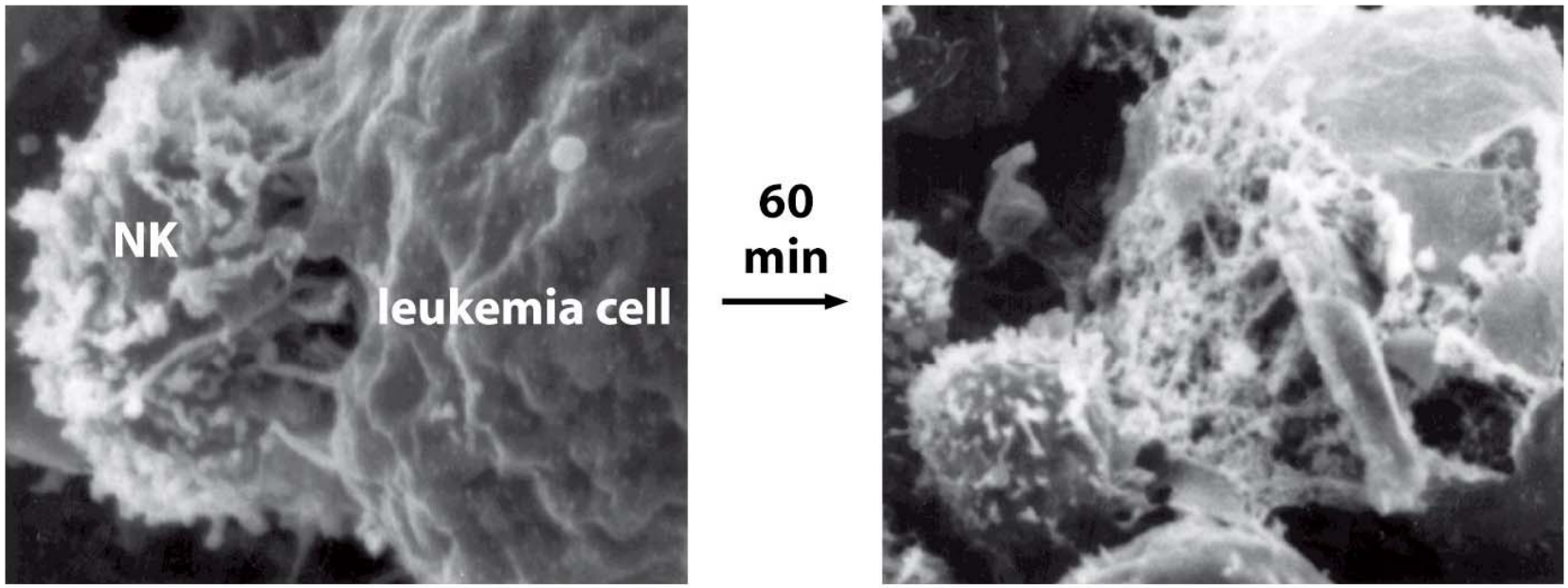
Development of the Innate Immune Response

Innate Immunity

- No previous exposure to antigen required
- 99% of animal species have only innate immunity
- Innate immune cells do NOT recognize specific antigens
- Innate immune cells recognize molecular patterns present on infectious agents (or transformed cells) that are not present on normal cells

Natural Killer Cells

- Kill cells coated with antibodies
- Kill cells that do not display normal levels of MHC I on the surface (e.g., virus-infected cells, cancer cells)
- Release cytokines to recruit other immune cells including macrophages



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The Immune System

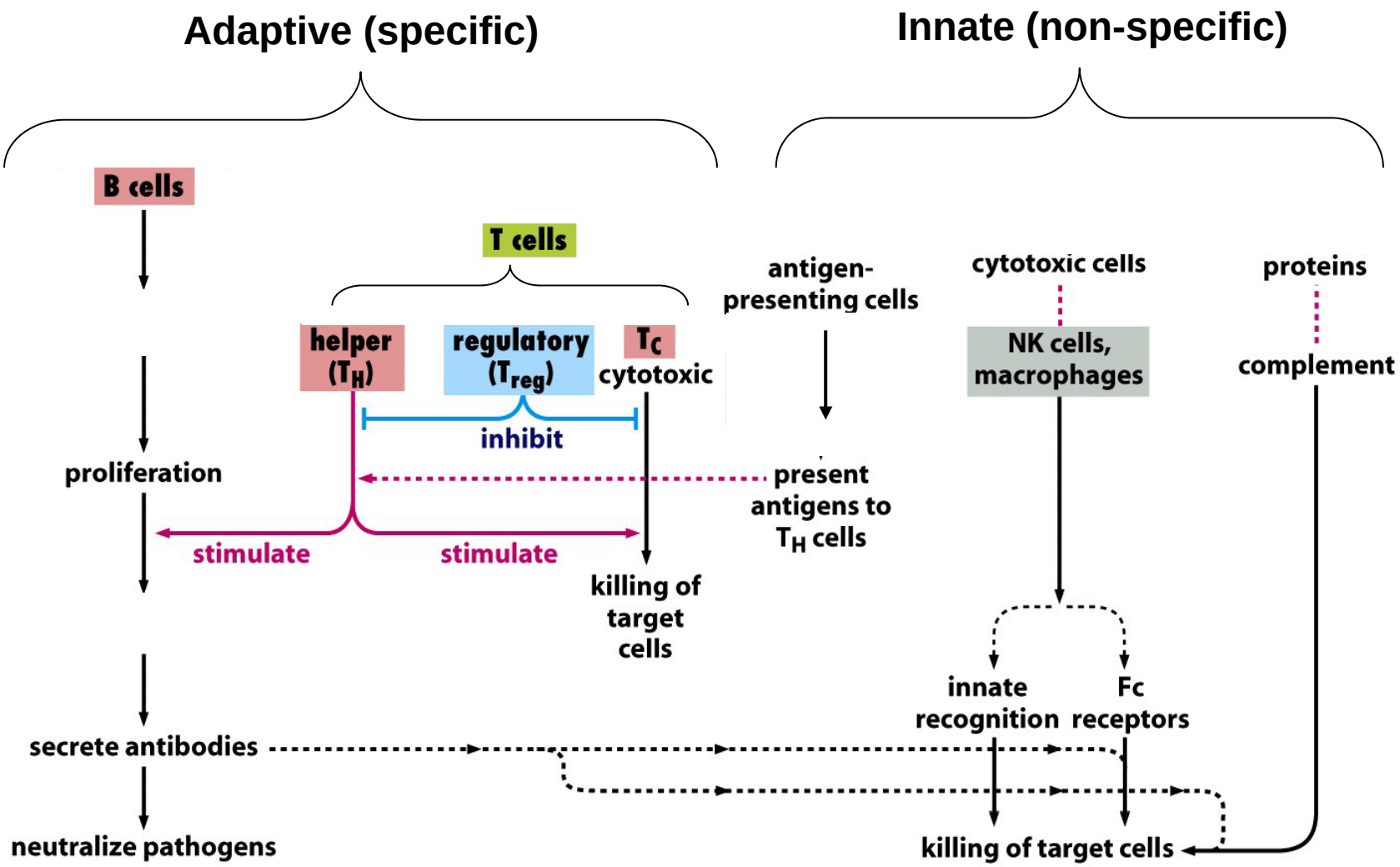


Figure 15-14 The Biology of Cancer (© Garland Science 2007)

Are Cancer Cells Recognized and Eliminated by the Immune System?

- **Immunosurveillance Theory**

Other Data in Support of Immunosurveillance

- Mice that can't produce perforin (no T_c) have more spontaneous tumors
- Mice with severely compromised immune system have more spontaneous and chemically induced tumors
- Immunocompromised individuals (humans) more susceptible to cancer than general population

Immune Cells at the Tumor Site

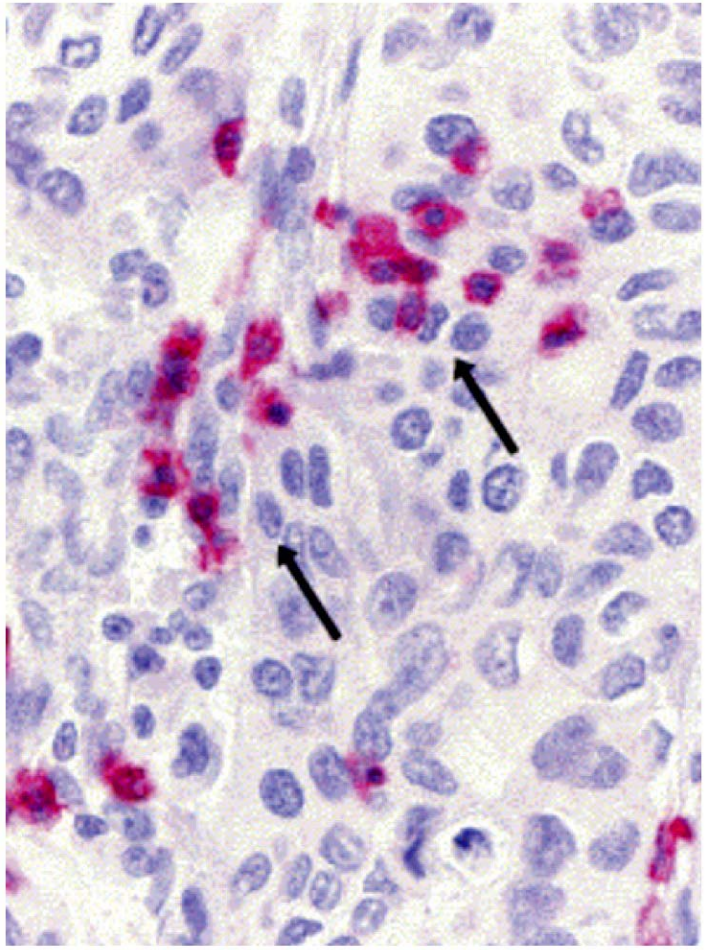


Figure 15-21c The Biology of Cancer (© Garland Science 2007)

- Tumor-infiltrating lymphocytes (TILs)
- Other immune cells (e.g., macrophages)

Non-small cell lung carcinoma section, stained with antibody to CD8, a marker for CTLs (pink)

TILs in Initial Tumor Correlate with Improved Survival

Study of ovarian cancer patients treated by surgery and chemotherapy

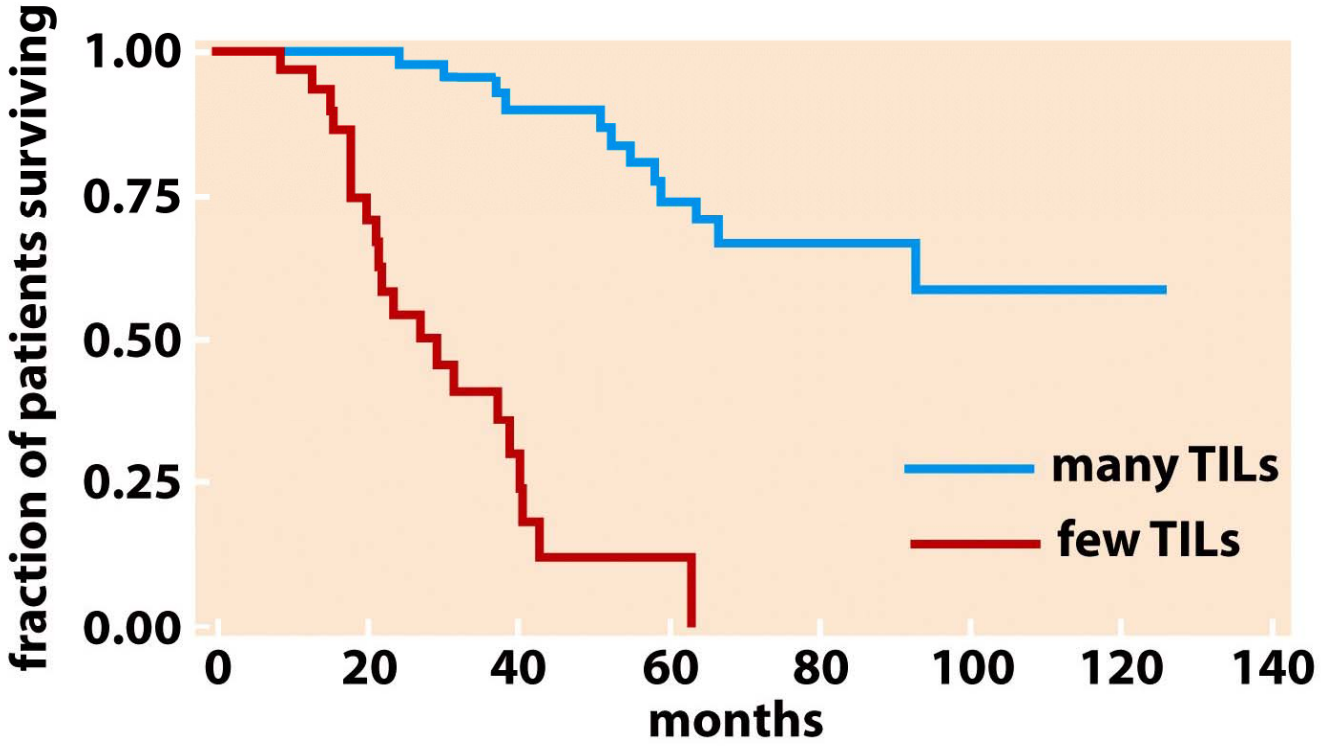


Figure 15-21d The Biology of Cancer (© Garland Science 2007)

How Might the Adaptive Immune System Distinguish Neoplastic from Normal?

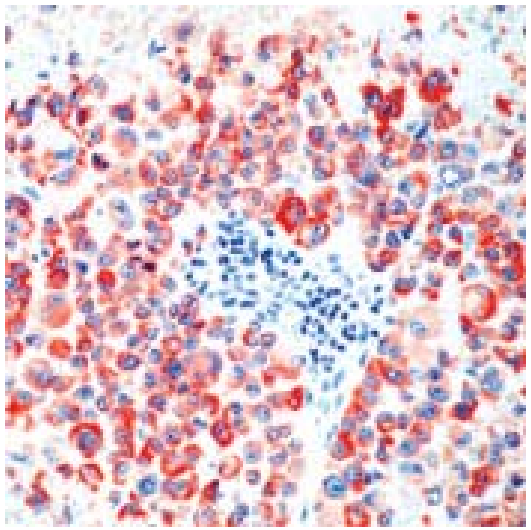
Recognition of tumor-associated antigens (TAAs):

- Tumor-specific proteins (e.g., viral proteins, products of mutated alleles that arise during tumor progression)
- Over-expressed proteins
- Embryonic or tissue-specific proteins

Tumor-Associated Antigens

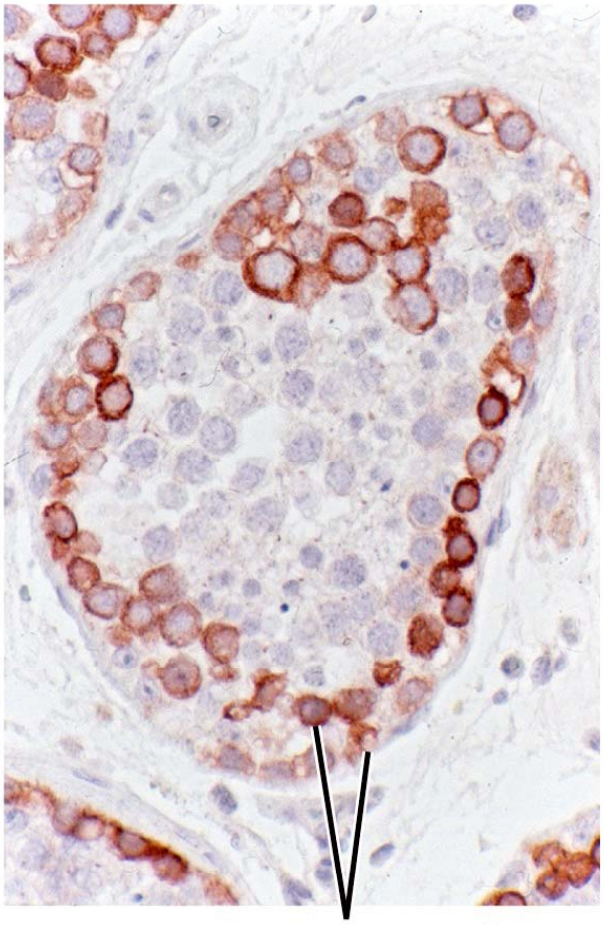
Melanoma is highly antigenic: expresses several TAAs

- Germ cell proteins (e.g., MAGE-1)
- Differentiation proteins (e.g., tyrosinase)



Human melanoma
Tyrosinase antibody staining
labvision.com

MAGE-1 antigen



Normal testes **spermatagonis**

Figure 15-24b The Biology of Cancer (© Garland Science 2007)

Chemically Induced Tumors are Antigenic

- Mice can be immunized with irradiated tumor cells, but they are only protected against cells from the same tumor

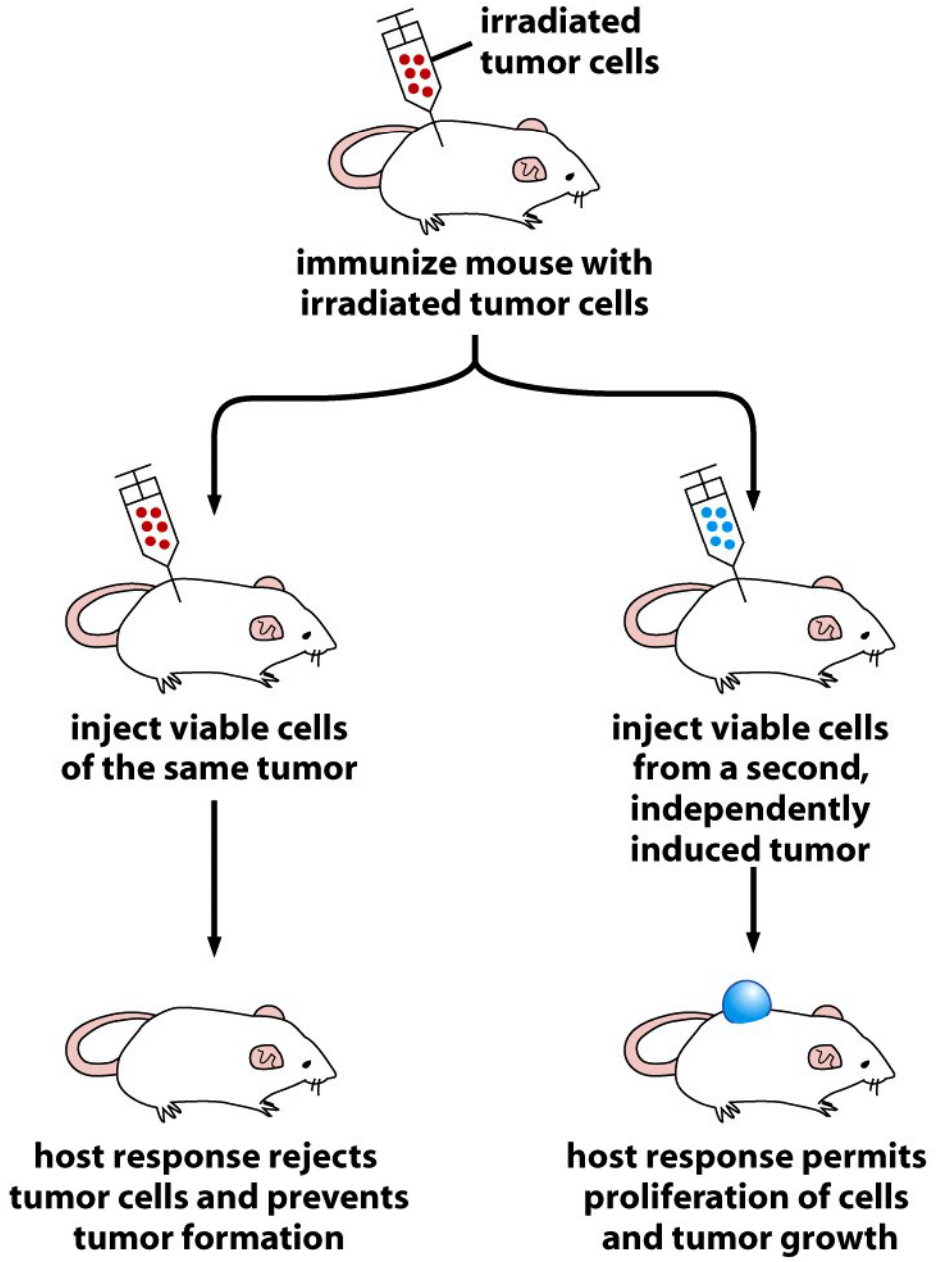


Figure 15-17 The Biology of Cancer (© Garland Science 2007)

TAAs Can Be Recognized by Immune System

- Immune attack on melanoma cells can also result in loss of normal melanocytes (loss of pigmentation = vitilago) due to shared antigens between normal and tumor cells
- Vitilago can be used as marker for improved survival of melanoma patients
- Other cancers: T cells and antibodies that recognize TAAs detected in patient's blood and tumor

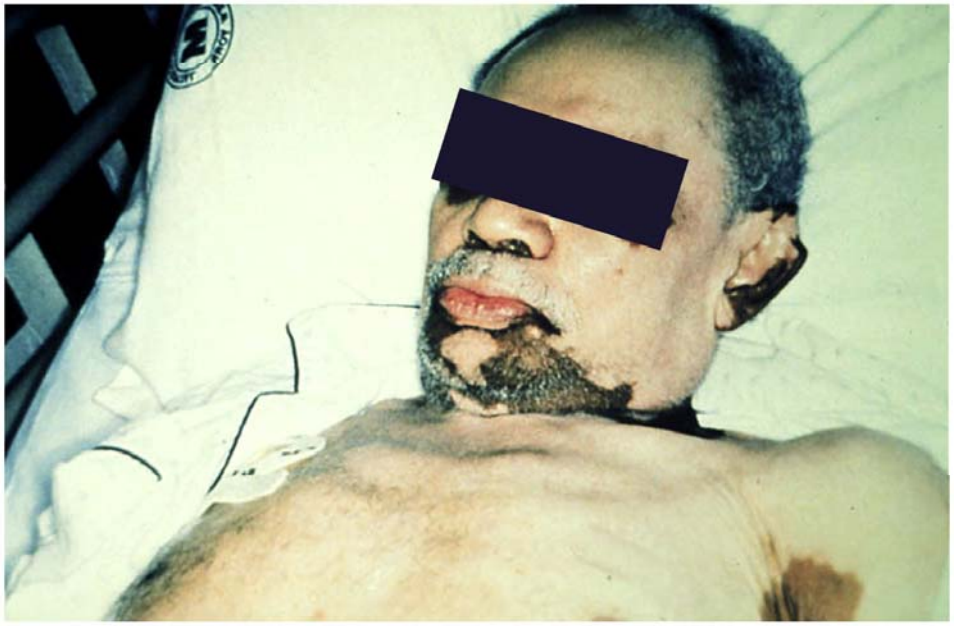


Figure 15-25 The Biology of Cancer (© Garland Science 2007)

Before onset of melanoma, this patient was dark skinned

Why aren't tumors completely eradicated by the immune system?

Possible explanations:

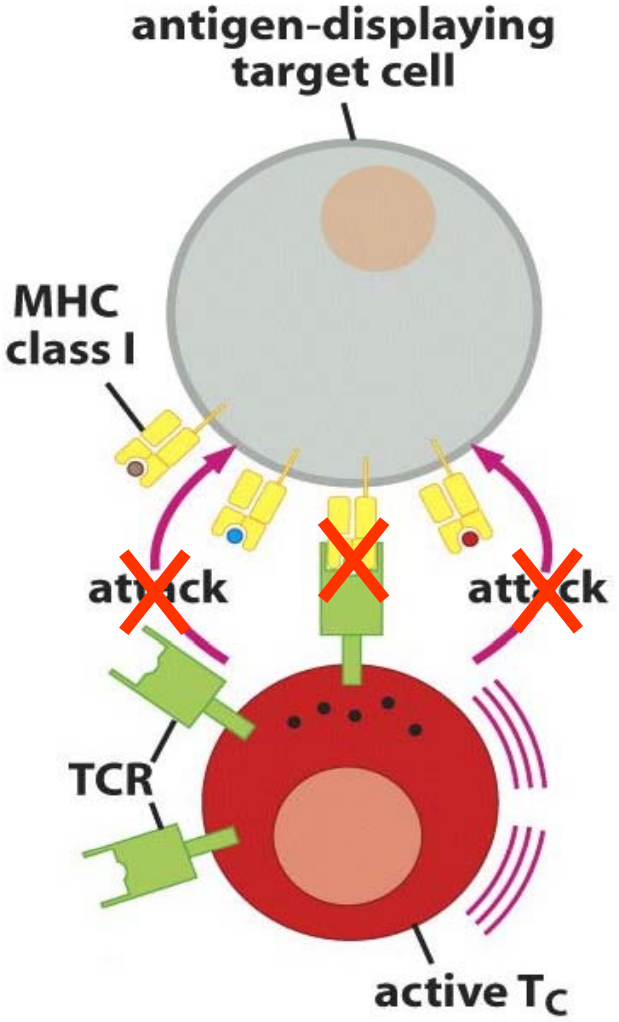
- Strongly antigenic tumors eliminated: weakly or non-antigenic tumors expand
- Selection for tumors with properties that allow them to evade the immune system

How Tumor Cells Evade the Immune System

- Altering tumor cell characteristics
  - decreased Ag presentation
  - anti-apoptotic state resistant to CTLs
- Suppressing the immune response
  - signal T cells to destruct (FasL)
  - secrete cytokines to prevent immune activity
  - neutralize complement
  - Attract T_{reg}s to tumor site
- "Hiding" from the immune response
  - immuno-privileged tissues
  - tumor architecture (necrotic pockets, poor vascularization)
- Outpacing the immune response

Loss of Antigen Presentation on Tumor Cells

- Selective advantage for tumor cells with low TAA expression
- More commonly: loss of MHC class I



Suppression of Class I MHC in Tumors

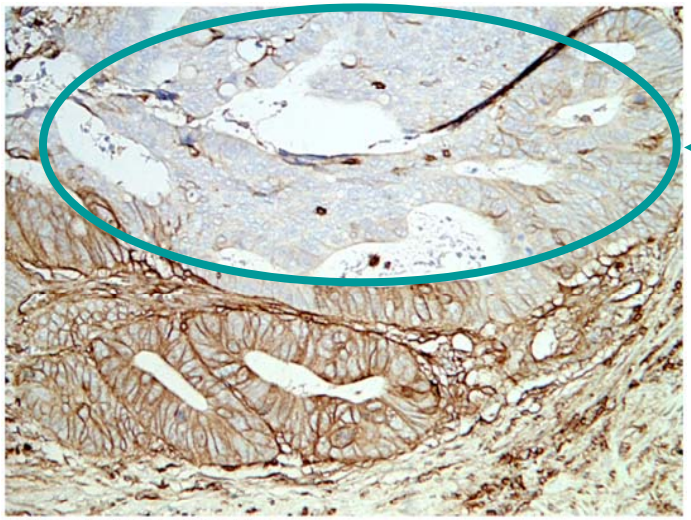


Figure 15-27 The Biology of Cancer (© Garland Science 2007)

Part of colorectal tumor has lost expression of HLA-A (an MHC class I molecule, brown)

- Associated with more invasive and metastatic tumors:
  - 50% of aggressive breast cancers lack MHC I
  - Due to block in MHC I transcription or down-regulation of MHC I transport to cell surface

MHC I Transport to Cell Surface in Normal Cells

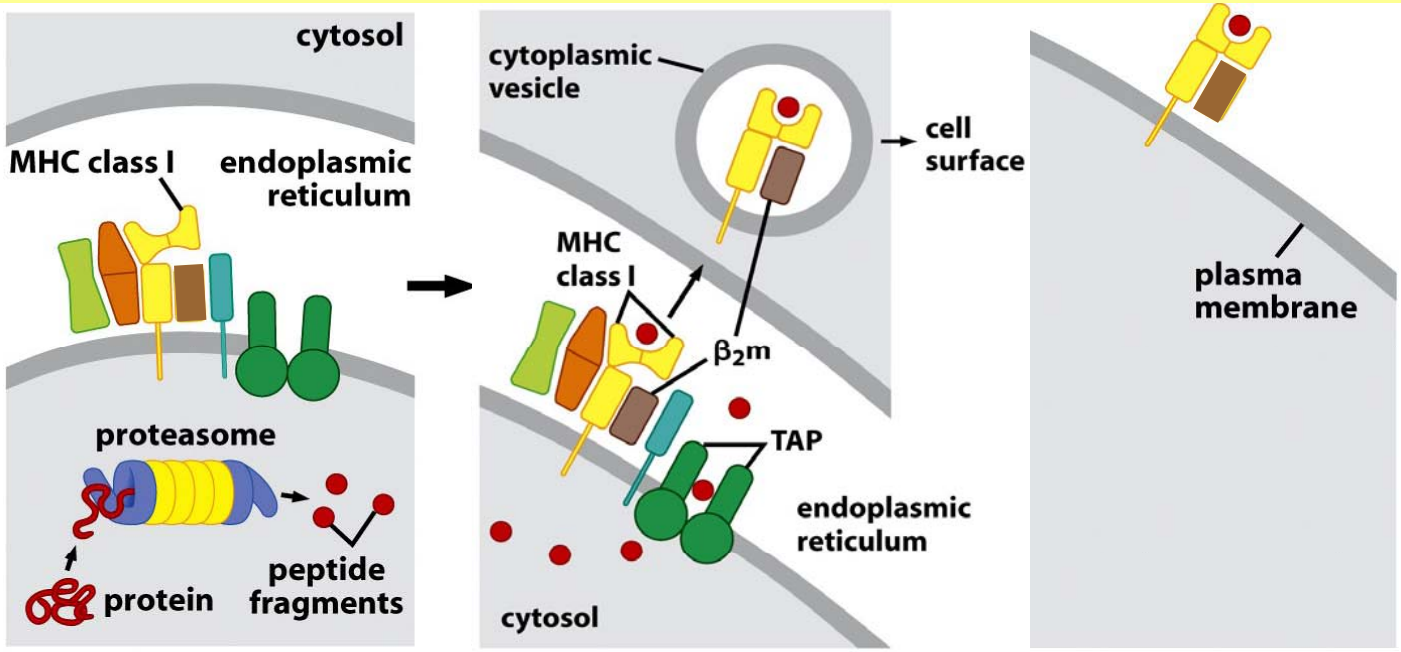


Figure 15-10a The Biology of Cancer (© Garland Science 2007)

Figure 15-28a The Biology of Cancer (© Garland Science 2007)

- TAP (transporter associated with antigen presentation) required for loading peptide on MHC
- $\beta_2$microglobulin (β_2m) escorts MHC-peptide complex to surface

➢ Loss of either of these prevents immune recognition

β_2Microglobulin Absent in many Colorectal Carcinoma Cells

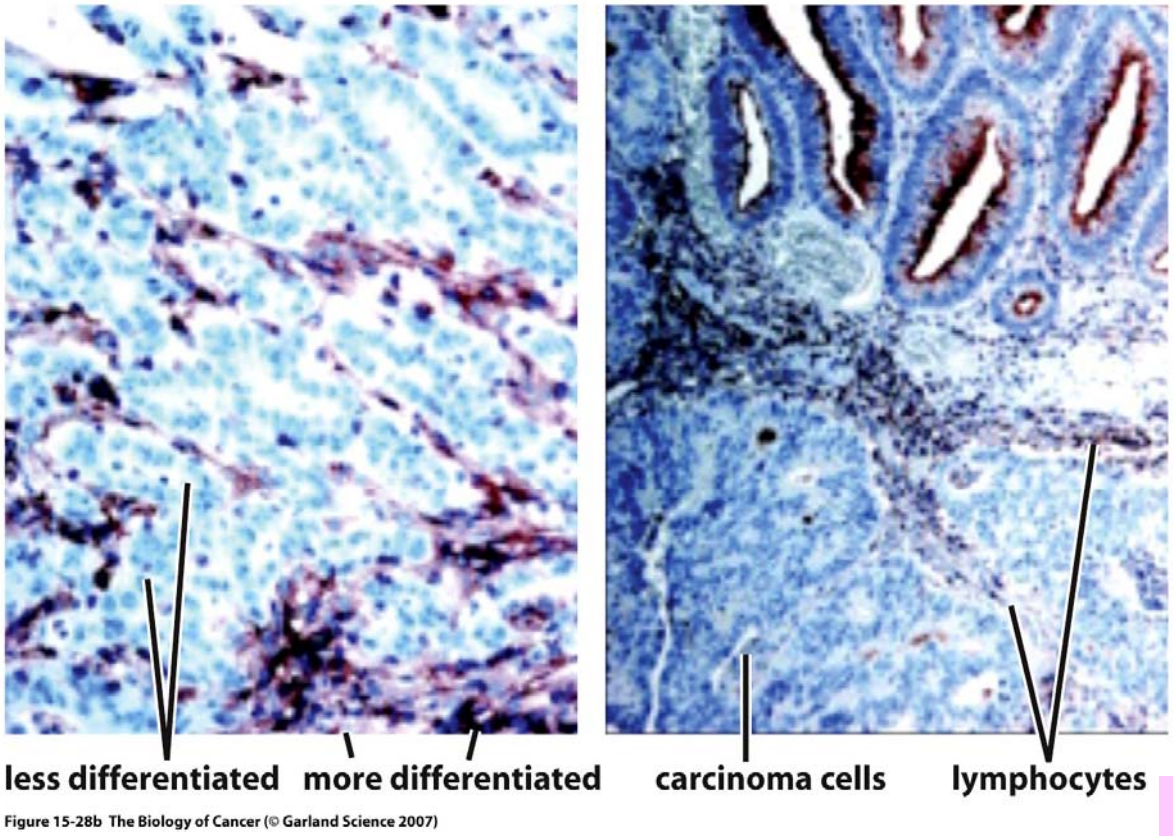


Figure 15-28b The Biology of Cancer (© Garland Science 2007)

Reddish purple: $\beta_2$microglobulin

low $\beta_2$microglobulin

↓

reduces MHC on surface

↓

reduces TAA presentation

↓

reduces recognition by CTL

➢But: low MHC class I makes tumor cells susceptible to NK attack

Tumor Cells Attack Immune Cells: FasL Expression

- Tumor cells secrete soluble FasL that induces apoptosis of nearby lymphocytes (and other non-tumor cells)
- Tumor cells resistant to apoptosis

Immunostaining of melanoma:
Blue: nuclei; red: FasL in vesicles

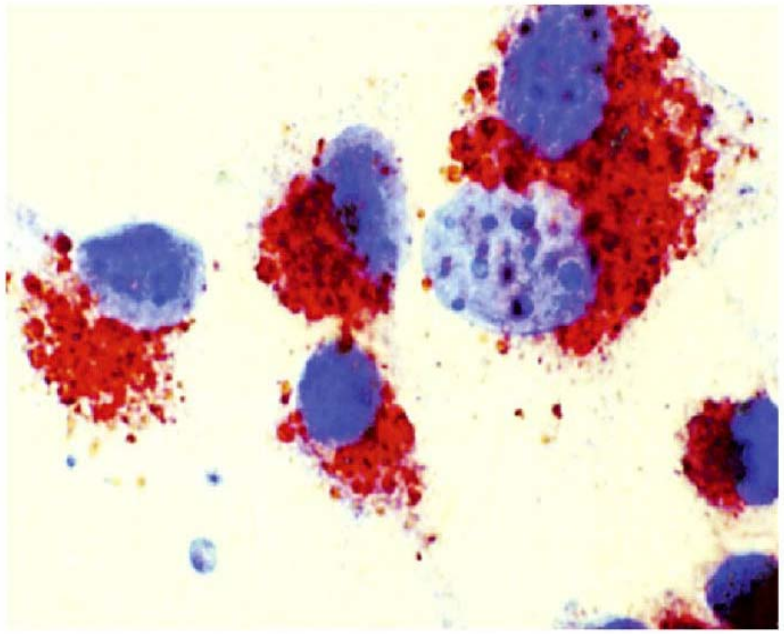
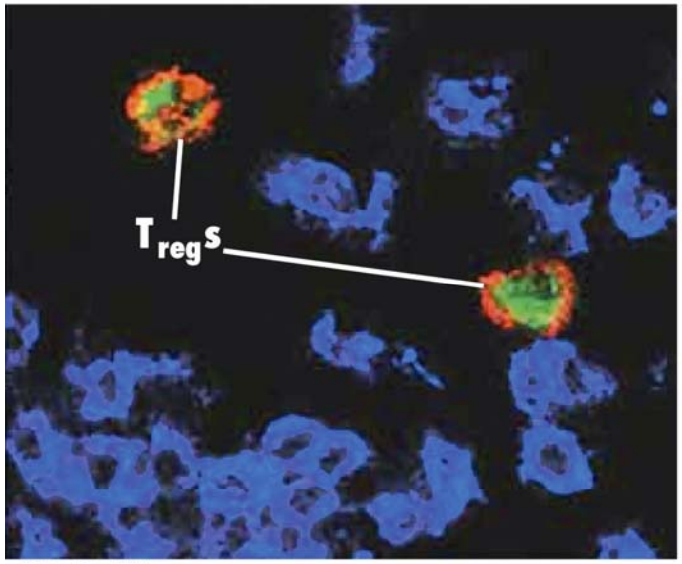


Figure 15-31a The Biology of Cancer (© Garland Science 2007)

Cancer Cells Attract T_{reg} Cells to Suppress Immune Response

- T_{reg}: antigen-specific T cell that inhibits (or kills) helper and cytotoxic T cells specific for the same antigen as the T_{reg}
- Chemokine (CCL22) secreted by tumor cells attracts T_{reg}s to the tumor site



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Fluid surrounding ovarian tumor (ascites) from ovarian cancer patient
Red, green: Treg markers
Blue: CTL marker

Number of Tumor-Infiltrating T_{reg} Cells Predicts Survival

- Number of T_{reg}s increases by several fold in cancer patients
- T_{reg}s found among the TILs

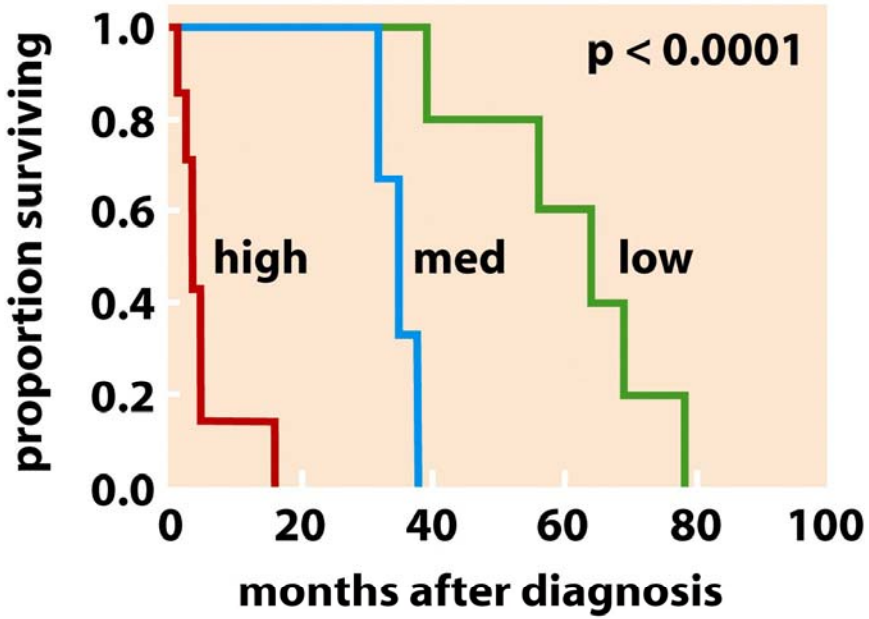


Figure 15-34c The Biology of Cancer (© Garland Science 2007)

Concentration (low, med, high) of tumor-infiltrating T_{reg}s in tumor sections of advanced ovarian carcinoma patients

➢ Low [T_{reg}] = improved survival

Can the Immune System be Manipulated to Target Cancer?

Cancer Immunotherapy: Active vs. Passive Immunization

- Active:
  - Requires active (or activateable) immune system in patient
  - Stimulation of immune system with cytokines or vaccines to produce antibodies or to activate T cells
  - Note: vaccines can be prophylactic (preventative) or therapeutic
- Passive:
  - Does not require active immune system
  - Therapeutic antibodies or immune cells provided to patient
  - Examples: Monoclonal antibodies against TAAs, bone marrow transplants

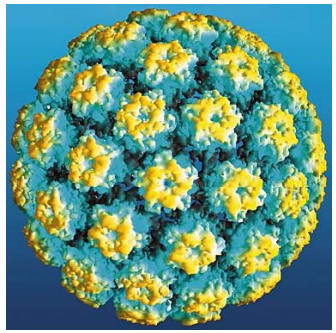
Cytokine Therapy

- Cytokine:
  - a protein ligand that binds to receptors on immune cells (usually) and activates that receptor's signaling pathway
  - can cause proliferation, differentiation, activation, or death depending on the target cell, receptor, and other pathways activated in the cell
- Effective in animal models: interleukins IL-2, IL-12, TNF, GM-CSF, and interferons
- Many induce toxicities
- Not generally very effective as single agents: greatest response in combination with other drugs

Prophylactic (Preventative) Vaccines

- Immunize against cancer-associated virus infections

- HPV and cervical cancer
  - In 2007, estimated 1,350 new cases of cervical cancer and 390 cervical cancer-related deaths in Canada
  - >99% of cervical carcinomas associated w. HPV
  - HPV vaccine (Gardasil, Cervarix) approved 2010, protects against two high risk types of HPV (16 and 18) (Gardasil also two low risk types)
  - Vaccine prevents nearly 100% of the precancerous lesions in the cervix associated with persistent infection with HPV type 16 and 18
  - Protects $\geq$ 5 years, few side effects



- HepC, HepB vaccines under investigation
- Why not more??

Therapeutic Cancer Vaccines

➢ To treat patients who already have cancer

- Types of defined tumor antigens under investigation:
  - Normal adult differentiation antigens: tyrosinase
  - “Tumor-specific” antigens: MAGE, telomerase
  - Over-expressed proteins: HER2, PSA (prostate specific antigen)
  - Mutated oncogenes: ras
- Undefined tumor antigens (tumor lysates)

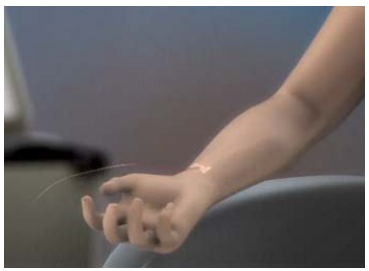
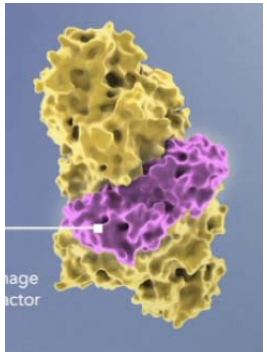
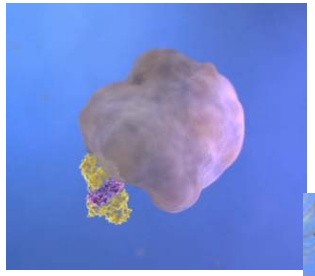
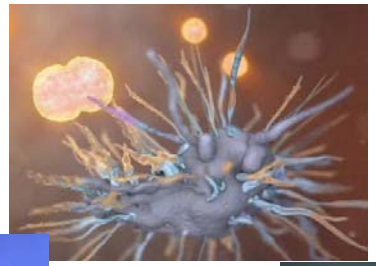
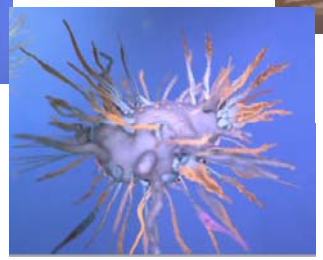
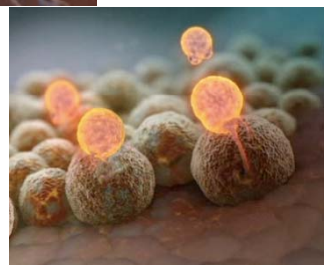
Immunization Strategies

Antigens can be administered several ways:

- Tumor cell based vaccine (undefined antigens)
- Tumor antigen/peptide based vaccine
- Genetic vaccine (DNA or RNA) encoding a tumor antigen/peptide injected *in vivo*
- Dendritic cells (antigen presenting cells) displaying immunogenic tumor cell peptides

Cancer Vaccines in the Clinic

- Over 100 clinical trials using cancer vaccines
- Several in phase 3, including melanoma trials with gp100 plus cytokine therapy
- In 2010, FDA approved a vaccine for advanced prostate cancer consisting of DCs treated with the TAA prostatic acid phosphatase protein fused with the cytokine GM-CSF (Provenge, Dendreon) based on several months survival benefit

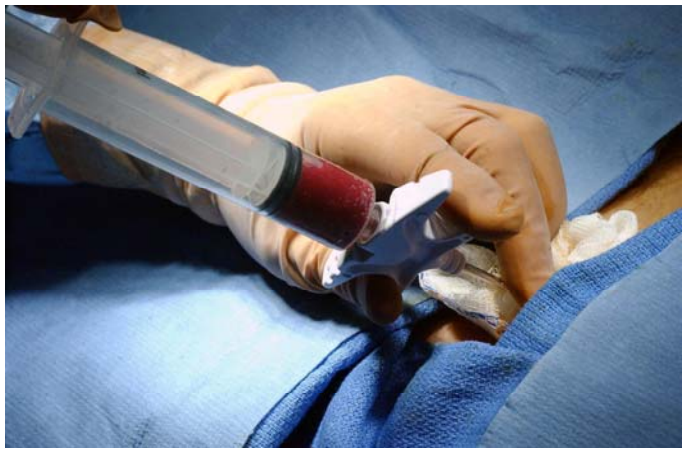







http://www.provenge.com/hcp/mechanism-of-action.aspx

Passive Immunization: Adoptive Transfer of Immune Cells to Cancer Patients

Bone marrow transplantation for hematopoietic malignancies:

- Leukemia and lymphoma (esp. CML) patients treated with drugs or radiation to eliminate their blood cell precursors, including all of the cancer cells
  - Bone marrow from donor is transplanted to patient to reconstitute a normal immune system (and RBC precursors)
  - Therapeutic effect likely due to immune cells of donor attacking residual tumor cells in patient



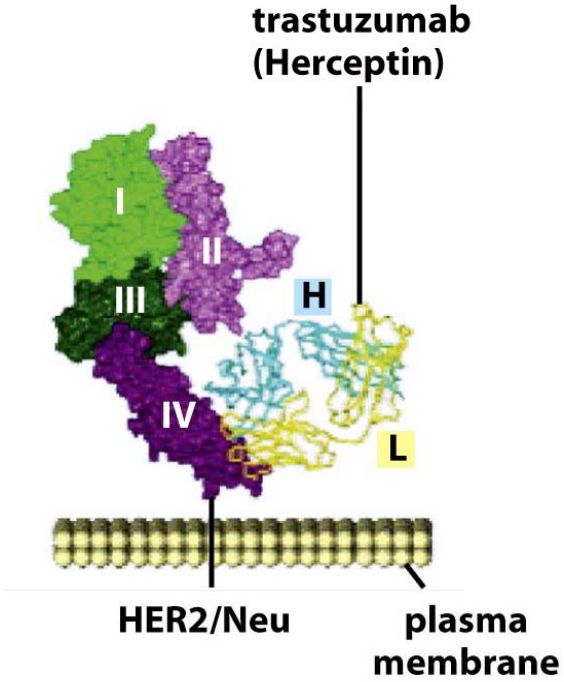
BM cells drawn from donor **Wikipedia**

Passive Immunization: Monoclonal Antibody Therapy

- Blocks activity of tumor-specific proteins
- Induces complement-mediated lysis of tumor cells, and/or
- Carries cytotoxic agent to tumor

Herceptin

- Herceptin (trastuzumab) is a monoclonal antibody specific for HER2 (= ErbB2 = Neu)
- HER2 is over-expressed (3-100X) on the surface of 30% of breast cancers



Weinberg, from Fig 15.38

Clinical Response to Herceptin

- Used in combination with chemotherapy for HER2+ patients with **advanced breast cancer** (compared to chemotherapy alone)
  - Slows progression of disease (7.4 vs. 4.6 months)
  - Lower death rate (22 vs. 36%)
  - Longer overall survival (25 vs. 20 months)

- Used in combination with surgery and chemotherapy for HER2+ patients with **early stage breast cancer** (compared to surgery and chemotherapy)
  - 15% relapse at 4 years, compared to 33%

One Mechanism of Herceptin Anti-Tumor Activity

- Antibody-dependent killing by NK cells

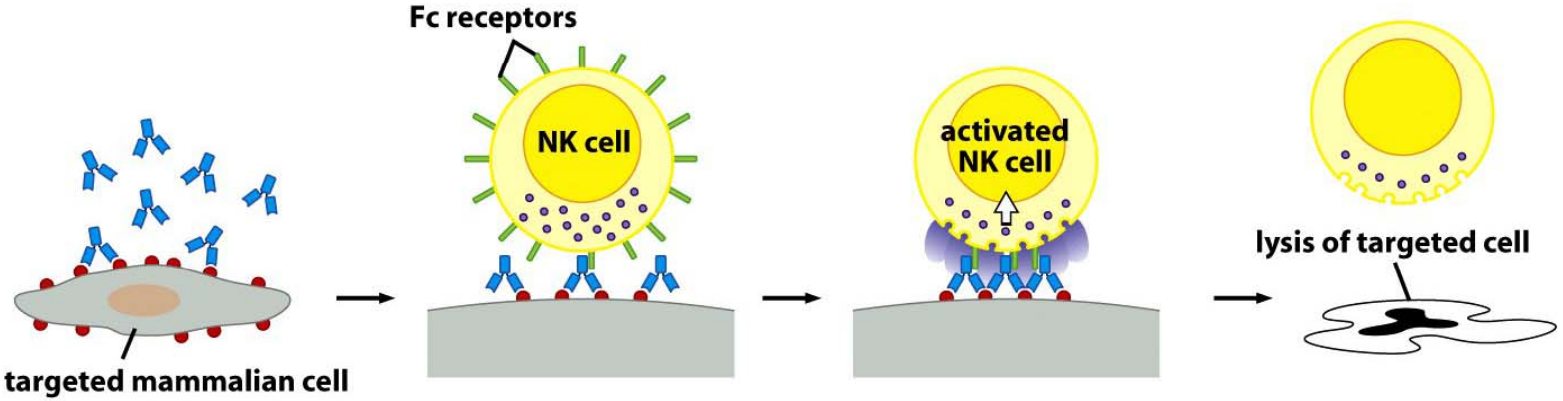


Figure 15-3b The Biology of Cancer (© Garland Science 2007)

- Mice deleted for a critical Fc receptor show reduced herceptin-dependent killing of tumor cells

Herceptin Induces HER2 Degradation, Minimizing Downstream Signaling

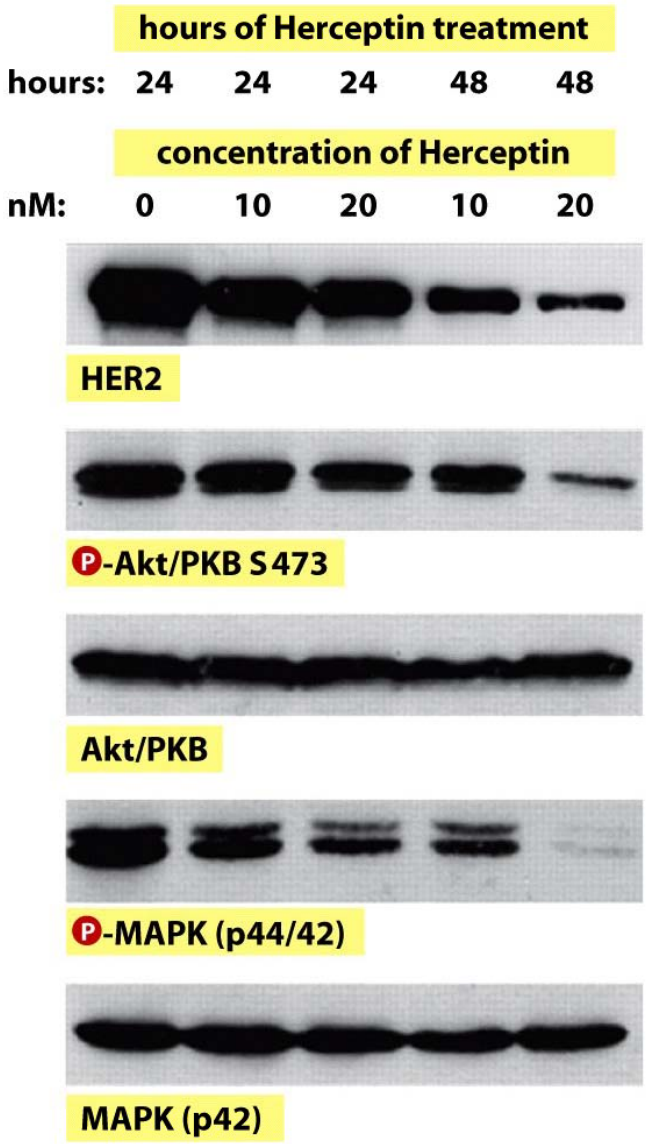


Figure 15-37a The Biology of Cancer (© Garland Science 2007)

- *In vitro* studies → no immune cells required
- Herceptin binding to HER2 induces internalization and degradation of receptor
- Loss of HER2 leads to decreased AKT and MAPK signaling

immunoblot

Herceptin Blocks Cleavage of Extracellular Domain of HER2

→ blocks constitutive activation

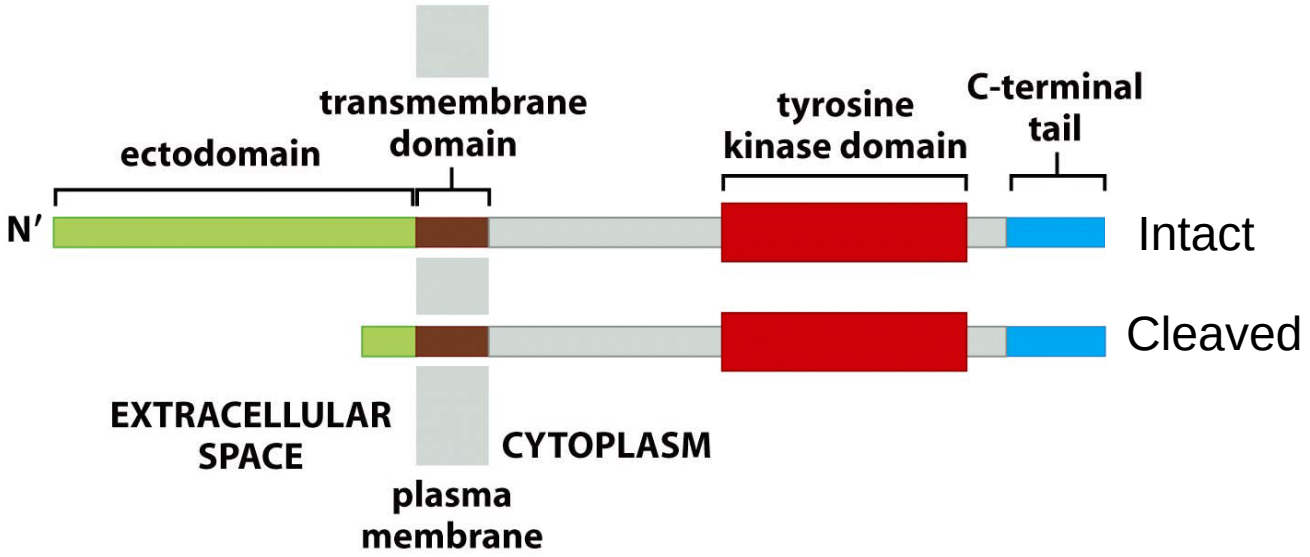


Figure 5-11 The Biology of Cancer (© Garland Science 2007)

Reduced Signaling following Herceptin Treatment Sensitizes Tumor Cells to Radiation

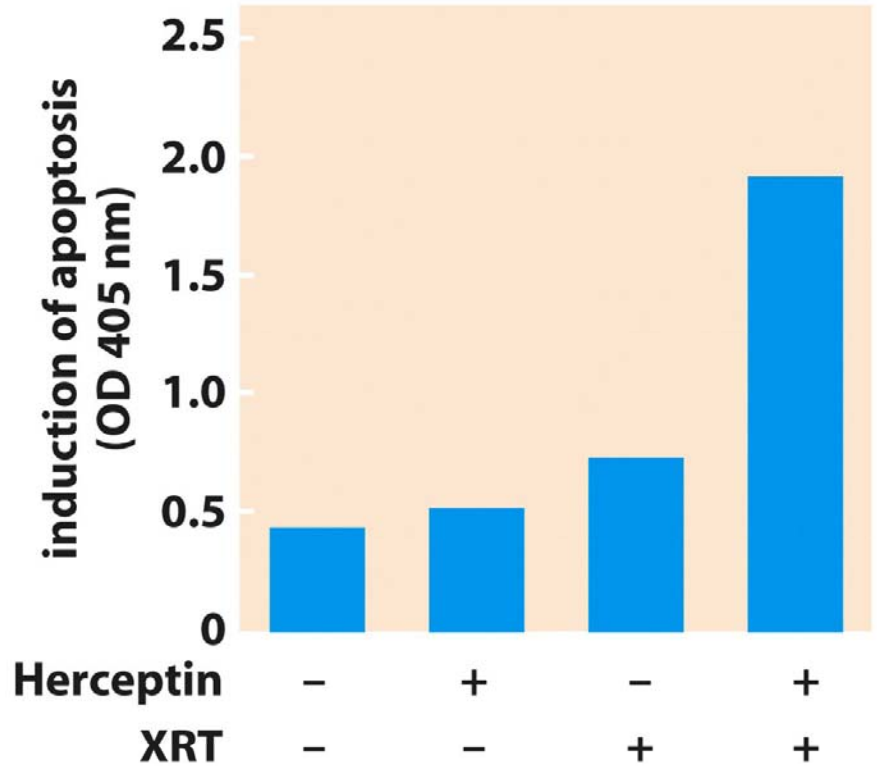


Figure 15-37b The Biology of Cancer (© Garland Science 2007)

- Breast cancer cells were treated with Herceptin, with X-rays (XRT), or with both, then assayed for apoptosis

➢ Increased apoptosis when combined

Rituxan Treatment for B-cell Malignancies

- Rituxan (rituximab) is a monoclonal antibody that binds to CD20, a surface marker of B-cells
- Non-Hodgkin's lymphoma (NHL):
  - 5^{th} and 6^{th} most common cause of cancer deaths in North American males and females, respectively
  - >90% of B-cell NHLs express CD20
  - Rituxan effective in treating relapsed (recurring) and refractory (unresponsive) NHL
- By 2003, rituxan used to treat >500,000 cancer patients: very effective in combination with standard treatment (40% improvement over standard alone)
  - Stabilizes disease, prolongs survival, does not cure
- Mechanism not precisely understood

Other Antibody Therapies

- Antibody targeting the EGF receptor (Erbitux) approved by FDA in 2004
- Bevacizumab (Avastin) binds to VEGF depriving the tumor of its blood supply

➢ In general, on their own, these therapies prolong life by several months, or even a few years. In combination with other drugs, much better.

- Monoclonal antibodies specific for receptors on tumor cells coupled to toxins (immunotoxin) or radioactive molecules, are also promising therapies

Summary

- Immune system classification: adaptive (antigen-specific) response and innate (non-specific) response
- Almost any cell can display processed intracellular antigens via MHC I for recognition by antigen-specific cytotoxic T cells.
- The immune system must be tolerant to normal proteins (mediated in part by T_{reg}s).
- Tumor associated antigens can be recognized by immune system.
- Tumors adapt to evade immune clearance by mechanisms that either prevent recognition, or that directly attack immune mediators.
- Monoclonal antibody therapies for cancer show promise, especially herceptin, rituxan.
- Cancer vaccines and other immunotherapeutics in development