Tumor Immunology and Immunotherapy

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• 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
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

Blue: antibodies, red: antigens

Figure 15-3b The Biology of Cancer (© Garland Science 2007)
Antibody-Mediated Elimination of Target Cells by Complement

- 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
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
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<sub>c</sub>)

- Activated CTL (= T<sub>c</sub>) recognizes its specific peptide-MHC complex on the target cell
- The T<sub>c</sub> 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

Figure 15-4b The Biology of Cancer (© Garland Science 2007)
Fas Death Receptor Activation

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

Figure 15-12e The Biology of Cancer (© Garland Science 2007)
The Immune System

Adaptive (specific)

- B cells
- T cells
  - helper (T_H)
  - regulatory (T_reg)
  - cytotoxic (T_c)

Innate (non-specific)

- antigen-presenting cells
- cytotoxic cells
- NK cells, macrophages
- proteins
- complement

proliferation

stimulate

inhibit

stimulate

killing of target cells

secrete antibodies

neutralize pathogens

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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 Tc) 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

- 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

![Graph showing survival rates with different TIL levels](image-url)
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
Melanoma is highly antigenic: expresses several TAAs

- Germ cell proteins (e.g., MAGE-1)
- Differentiation proteins (e.g., tyrosinase)
Chemically Induced Tumors are Antigenic

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

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

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

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

> Loss of either of these prevents immune recognition
\( \beta_2 \) Microglobulin Absent in many Colorectal Carcinoma Cells

- Low \( \beta_2 \) microglobulin reduces MHC on surface
- Low \( \beta_2 \) microglobulin reduces TAA presentation
- Low \( \beta_2 \) microglobulin reduces recognition by CTL

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

Reddish purple: \( \beta_2 \) microglobulin
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\textsubscript{reg} Cells to Suppress Immune Response

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

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

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?

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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 ≥ 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

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
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

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

Figure 15-3b The Biology of Cancer (© Garland Science 2007)
Herceptin Induces HER2 Degradation, Minimizing Downstream Signaling

- *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

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

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Herceptin Blocks Cleavage of Extracellular Domain of HER2

→ blocks constitutive activation
Reduced Signaling following Herceptin Treatment Sensitizes Tumor Cells to Radiation

- 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):
  - 5th and 6th 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
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_{regs}).
- 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