Primer on Tumor Immunology

International Society for Biological Therapy of Cancer

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Outline: Primer on Tumor Immunology

- T Cell Receptors
- T Cell Biology
- Tumor immunology
Organization of Immune System

Innate Immunity

Adaptive Immunity

Immune System

Humoral (B Cells)  Cell mediated (T Cells)
The Course of Induction of Innate and Adaptive Immunity

Janeway, Immunobiology
Hallmarks of the Adaptive Immune Response

1. Increasing specificity

2. Rechallenge: response is more rapid and more robust

3. Memory: Selected T cells persist for years
Innate and Acquired Immunity

Extracellular Organisms
- Humoral Immunity
  - Antibody
  - B Cells
  - Granulocyte
  - Complement

Innate Immunity

Acquired Immunity

Intracellular Organisms
- Cell-mediated Immunity
  - NK Cells
  - T Cells
  - Macrophage
  - Cytokines
T Cell Differentiation: Multiple Mature Subsets

Leukocytes

Monocytes & Macrophages

Circulating Dendritic cells

Granulocytes
- Basophils
- Eosinophils
- Neutrophils

Platelets

Erythrocytes

Lymphocytes

B cells

T cells
- CD5+
- CD5−

NK cells

iNKT

TH

T reg

TC/S

γδ T cells

Naive
Memory

Naive
Memory
Naive T cell

Activated Memory (Effector) Cells

Resting Memory Cells

Primary encounter to Ag: signal to differentiate

Secondary encounter to Ag: signal to divide

Immune response completes; antigen disappears. Most cells die by apoptosis

Bone Marrow

Thymus

Intestine

iIEL cells

Naive T cell

Generation of T cells
T Cell Biology

• T cells develop in the thymus
  – TCR rearrangement

• T cells belong to the class of cells that have capacity to enter and exit the cell cycle

• Mature T cells divide in secondary lymph tissue
  – Spleen, lymph nodes
  – Do not divide in peripheral blood
Principles of T Cell Activation

- T cells are activated by antigen presenting cells (APC)
- Any cell that expresses MHCI or MHCII can activate T cells
- Naïve T cells have more stringent requirements: only a DC can do the job
T Cell Activation

- **Immunosurveillance**: DC / APC take up Ag in periphery
- **T cell division and clonal expansion** occurs in lymph nodes
- **Geographic and temporal control**
  - Naïve T cells reside in lymph nodes
  - DC bring antigen to the lymph node

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Janeway, Immunobiology
The Three Laws of Immunology

1. The Immune System is Capable of Recognizing a Virtually Unlimited Array of Specific Structures (Universality)

2. The Response to Self-Antigens is Eliminated or Controlled (Tolerance)

3. The Response is Appropriate to the Inducing Pathogen (Appropriateness)

Adapted from W. E. Paul, M.D., Editor, Fundamental Immunology
T Cell Activation

Invariant Costimulatory Receptors

Clonally rearranged TCR

Janeway, Immunobiology
The First Law - *Universality*

Lymphocytes are the specific cells of the immune system.

Each one is different in that it can recognize a different structure.

Lymphocytes use a cell surface receptor for this recognition.

Humans have $\sim 10^{12}$ lymphocytes.
Clonal Selection

Antigen-driven Deletion

Antigen-driven Expansion
Genetic Basis of Specificity

Somatic Mutation

“Germ-Line” Gene

“Mutant” Genes

Germ-Line Encoded
Recombination of V, D and J Elements to Construct an H Chain Gene

At V & D and D & J junctions there is joining imprecision, deletion of nucleotides and addition of untemplated nucleotides; the result is a virtual random peptide generator creating an enormous potential repertoire of H chains.
T Cells Resemble Unicellular Organisms

- Individual T cells are capable of expanding or contracting in number depending on whether they have a selection advantage (recognition of antigen).

- The immune system evolves *somatically* and thus provides a mechanism to deal with the rapidly evolving microbial world.

- The TCR repertoire is continually evolving
T Lymphocyte Homeostasis

T Cell Production
Thymic
Extrathymic

Pathogen or
Tumor Induced
Demise

Natural Demise
apoptosis

Repertoire $\sim 10^9$
T lymphocyte mass $\sim 10^{12}$
Life-span: months to years
The Second Law – *Tolerance*

**T Cells**

- Negative selection in the thymus
- Clonal elimination in the periphery
  - Encounter with cognate antigens on immature dendritic cells
- Clonal anergy
  - Encounter with cognate antigens in the absence of co-stimulation inactivates T cells
- Regulatory/suppressor T cells
  - Is this a tolerance mechanism or a mechanism to control the magnitude of immune responses
Clonal Elimination; “Classical View”

Antigen-driven Deletion; Occurs during T Cell Development

Antigen-driven Expansion; Occurs when T cell is Mature
Self Recognition: Both Essential and Dangerous

• The immune system plays a “dangerous” game.

• It relies on self-recognition both to create the repertoire of T cells that are allowed to populate the periphery and for the survival of these cells and yet it needs to control self recognition to avoid a dangerous attack on self-tissues.

• A current view is that the border between these is quantitative - that “good” self recognition is low affinity and results only in survival signals while dangerous self-recognition is high affinity and results in expansion and differentiation into effector cells.
B Cells & T Cells Deal with Different Antigenic Universes

B Cells Recognize the Universe of 3-dimensional Structures

T Cells Recognize the Universe Of Peptide/MHC Complexes
The CD4 T Cell Antigenic Universe

T Cell Receptors Recognize Peptide/MHC Complexes
The CD8 T Cell Antigenic Universe

T Cell Receptors Recognize Peptide/MHC Complexes

Intracellular Proteolysis

Peptide

Class I MHC Molecule

Antigen

Antigen-Presenting Cell

CD8 T Cell

T Cell Receptor
Pivotal Roles of CD4 Cells

- CD40L
- IL-2
- IL-4
- CD40L

- Activated Macrophages
- CD8 Cytotoxic T Cells
- B Cells
Tolerance is Controlled by CD4 Cells
Three Faces of CD4 T Cells

**T-bet**
- Th1: Fight Intracellular Pathogens

**Gata-3**
- Th2: Fight Extracellular Pathogens

**FoxP3**
- Treg: Silences Effector T Cells
  - Accumulate at tumor microenvironment
Mechanisms of Tolerance Induction

**Normal**
- Antigen-presenting cell
- CD80
- MHC
- Peptide
- Anatomical barrier
- T-cell receptor
- CD28
- CD3
- Activated T cell

**Immunologic Ignorance**
- Fas ligand
- Fas
- CD152
- Interleukin-10
- TGF-β
- Regulatory T cell

**Deletion**
- Apoptosis

**Inhibition**
- No activation

**Suppression**
- No activation

*NEJM 344: 655, 2001*
Autoimmunity

- Autoimmunity is the loss of tolerance to self antigens
- Many approaches to tumor therapy attempt to *break* tolerance to self antigens expressed on tumors
- Expected consequences: tissue specific autoimmunity
T Cell Activation: antigenicity vs immunogenicity

The concept of costimulation

**Antigen:APC T cell interaction: no costimulation:**
Result: T cell anergy, apoptosis or suppression (Treg cells)

**Antigen:APC T cell interaction: with costimulation:**
Result: T cell activation, clonal expansion, effector functions
The CD28 and B7 Receptor Families
CD28 Receptor Family - 1999

- **CD80 and CD86 on APC**
- **CTLA-4**
  - Inducible
  - Cell surface and intracellular localization
  - Negative costimulation of TCR-mediated signals
  - TGF-β secretion
- **CD28**
  - Constitutive
  - Cell surface localization
  - Strong costimulation of TCR-mediated signals
- **ICOSL (B7h) on APC**
- **ICOS**
  - Inducible
  - Cell surface localization
  - Role unknown
  - IL-10 secretion
The CD28 and B7 Receptor Families - 2005

APC  Ligand  Receptor  T cell

9p24  21q22  3q21

B7x  B7H3  PDL-2  PDL-1  ICOS-L

CD80  CD86

BTLA  PD-1  ICOS  CD152  CD28

2q33  2q37
The CD28 Family Homologies
Roles of CD28

- Induction and maintenance of cytokine and chemokine secretion
- Cell survival: bcl-X induction and promotes clonal expansion
- Enhanced telomerase activity
- Required for T cells to increase their glycolytic rate (PI3K and Akt)
- Down regulation of beta chemokine receptor expression
- Costimulation and “superagonists”
The CD28 Family

- Residues important for natural ligand binding
- Residues important for superagonist binding
- Unpaired Cysteine

SH2 Binding Domain
SH3 Binding Domain
ITIM
ITSM
GRB2 Binding Domain
CTLA4 Regulates T Cell Numbers

Negative roles

- CTLA-4-deficient mice die within 4 wk after birth due to a lymphoproliferative disorder of CD4 T cells
- Lymphoproliferative disease in the absence of CTLA-4 is not T cell autonomous: bone marrow chimeras producing CTLA-4-/- and normal T cells are healthy
- CTLA-4 recruits phosphatases to the TCR complex

Positive roles

- Increases beta chemokine receptor expression

*Science* 1995; 270: 985
CTLA4 Regulates T Cell Mass

CTLA4 -/-  CTLA4 +/+
• Polymorphisms of CTLA4 associated with multiple autoimmune disorders
  – Polymorphism in the 3’ UTR of CTLA4
  – Reduced production of a splice form encoding a molecule lacking the CD80/CD86 ligand-binding domain.
  – Mechanism: effects on CTLA4 signals or Treg?
Blocking Negative Costimulatory Receptor Function Can Lead to Autoimmunity

• Antagonistic CTLA4 antibody therapy in cancer patients leads to autoimmunity
  – Phan et al, PNAS 2003; 100: 8372
  – Multiple organs affected
  – Prominent target organ is GI tract: IBD like lesion
  – Symptoms resolve with corticosteroids
  – Augmented anti-tumor effects
Soluble Fusion Proteins of CTLA4 Can Treat Autoimmune Disorders

Rationale: soluble ligand for B7 that prevents B7:CD28 interaction.

Table 1 Selected CTLA4-Ig drugs in clinical trials

<table>
<thead>
<tr>
<th>Company</th>
<th>Indication</th>
<th>Drug</th>
<th>Stage of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bristol-Myers Squibb</td>
<td>Rheumatoid arthritis</td>
<td>CTLA4-Ig</td>
<td>Phase 3</td>
</tr>
<tr>
<td>Bristol-Myers Squibb</td>
<td>Multiple sclerosis</td>
<td>CTLA4-Ig</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Bristol-Myers Squibb</td>
<td>Kidney transplantation</td>
<td>LEA29Y</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Repligen</td>
<td>Multiple sclerosis</td>
<td>CTLA4-Ig (C, gamma4)</td>
<td>Phase 1/2</td>
</tr>
<tr>
<td>Bristol-Myers Squibb</td>
<td>Lupus</td>
<td>CTLA4-Ig</td>
<td>Phase 2 (pending)</td>
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Why are there so many costimulatory molecules?

Hypotheses:

1. Fine Tuning of the Immune Response
2. Tissue Specificity
3. Distinct signal requirements to trigger differentiation and drive activation
4. Instructional Role of DC in Generation of T cell Subsets

Generation of CD8 Memory T Cells

Esser MT, et al.

- **CD28**
- **CD107**
- **CD83**
- **IL-15**
- **IL-21?**
- **4-1BB**

**T naive**
- CD45RA+
- CCR7+
- CD62L+
- CD27+

**Naive**
- Short Activation
- Sites of infection
- TH1 or TH2 cytokines
- Perforin

**Effectors**
- CD45RA+
- CCR7-
- CD62L-
- CD27-

**T Effect Memory**
- CD45RA+ or CD45RO+
- CCR7-
- CD62L-
- CD27-
- Short Term Activation
- Periphery & Mucosae
- TH1 or TH2 cytokines
- Perforin

**T EM**
- T Central Memory
- CD45RA+ or CD45RO+
- CCR7+
- CD62L+
- CD27+
- Delayed Activation
- Skin & Lymph Nodes
- IL-2 + IL-10
- DC help (CD40L)

**T CM**
- > 90%
Tumor Immunology: Basic Concepts

- **Current Dogma**: “Tumor cells are antigenic but not immunogenic”
- **T cells** (T for “tumor and thymus”) mediate specific tumor rejection. Natural killer cells also have a role in tumor elimination.
- **B cells and antibody** appear to have little or no physiologic role in immune tumor elimination since these immune effector systems are best suited for extracellular antigens.
- “**Tumor Darwinism**”: Tumors have evolved sophisticated means to avoid immune detection.
Cancer Immunosurveillance Theory

• The T cell immune system almost certainly evolved as a defense mechanism to control viral infections; it definitely did not evolve to control tumors!

• Given the demonstration of tumor specific antigens, during the 1960s and 1970s, there was wide acceptance of the "immunosurveillance" model put forth by Lewis Thomas and MacFarlane Burnet.

• 1980s, SCID mice (T-NK+): had normal incidence of tumors: toss the theory!

• 2000, mice with deficient IFNgamma signaling have large increase in spontaneous tumors (Schreiber et al, Nature, 2001): resurrects theory.
Tumor Immunosurveillance

- Immunosurveillance: Compelling data from human patients indicates that cancer immunosurveillance acts as an extrinsic tumor suppressor
- Immunoediting: immune surveillance facilitates the outgrowth of tumors with reduced immunogenicity (Schreiber and Old)

## Enhanced susceptibility of immunodeficient mice to spontaneous and chemically induced tumors in immunosuppressed animals

<table>
<thead>
<tr>
<th>Technology</th>
<th>Immune status</th>
<th>Tumor susceptibility relative to wild type</th>
</tr>
</thead>
</table>
| RAG-2 
\( -/- \) | Lacks T, B, NKT cells | ↑ MCA-induced sarcomas; |
| RAG-2 
\( -/- \) \( \times \) STAT1 
\( -/- \) | Lacks T, B, NKT cells; IFN\( \gamma \)-, \( \alpha/\beta \)-insensitive | ↑ spontaneous intestinal neoplasia; ↑ MCA-induced sarcomas; ↑ spontaneous intestinal and mammary neoplasia |
| LMP2 
\( -/- \) | Lacks LMP2 subunit | ↑ Spontaneous uterine neoplasms |

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Mechanisms of Tumor Immune Evasion

**Immunogenicity**
- decreased peptide:MHC complexes
- decreased

**Tumor Induced Immune Suppression**
- e.g. TGFβ
- e.g. IL-10

**Antigenic Modulation**
- Selection for “loss variants”
Summary: Tumor Immunology

- **Basic principles of tumor immunology**
  - Know the three laws!
- **Cancer antigens: generally “self” antigens**
  - Implies immune system will be biased to *tolerogenic* responses
- **Tumor immunosurveillance and immunoediting**
- **Tumor induced immune suppression**
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