Tumor Immunity (Clynes)

Tumor Immunology

- Does it exist?
  i.e., does the immune system recognize and eradicate cancer cells? Is there any evidence for immunological surveillance (Burnett and Thomas)?
- How can the immune system recognize cancer if it is essentially self-tissue? (Tolerance)
- If it does not- can it be made to do so? (Immunization designed to Break Tolerance)

Where is the danger—the innate activator?

The Good News/Bad News Story

The immune system can destroy self-tissue quite effectively in autoimmunity, and in a tissue-specific (antigen-specific) manner: rheumatoid, hepatitis, pancreatitis (diabetes), vitiligo, ITP, AIHA, graft rejection etc. So, self-tissue destruction can be potent.

- Are there ongoing anti-tumor immune responses in patients with cancer?
  - Spontaneous remissions are rare but can occur, renal cell CA, melanoma, and are associated with anti-tumor Abs and CTLs.

  TIL cells (tumor infiltrating cells) include CTLs that recognize melanoma antigens/peptides (6/11 patients). But these CTLs were anergic: could not kill targets or produce γ-IFN.

  So...the good news is that immune recognition of tumor antigens occurs but the bad news is that this occurs without activation of immune effector responses.

More “good” news

Evidence for Immunological Surveillance in Man

Cancer Incidence Increases in Immunosuppressed

- Increased incidence of malignancies in HIV patients; EBV lymphoma, KS, squamous cell CA. But many of these are virally induced malignancies; this merely shows that eliminating a T cell response against viral antigens allows for the outgrowth of virally-transformed cells. Common variety neoplasms (colon, breast, prostate, lung, etc.) may be seen with increased frequency as HIV patients live with their disease longer.

- In transplant associated EBV lymphomas (presumably arise after the loss of EBV specific CTLs associated with T cell depleted allo-BMT. Cures are achievable by infusion of donor T cells (reconstitute CTL response). Again loss of an anti-viral response is implicated. (post-transplant patients are also at increased risk for melanoma and sarcoma).

Tumor Incidence

<table>
<thead>
<tr>
<th>Host: RAG-/-</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor: RAG-/- origin</td>
<td>WT</td>
</tr>
</tbody>
</table>

Tumor Incidence is Increased in MCA-treated Lymphocyte Deficient Mice

Tumor Size

<table>
<thead>
<tr>
<th>Host: WT</th>
<th>RAG-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor: WT origin</td>
<td>RAG-/- origin</td>
</tr>
</tbody>
</table>

Tumors which developed in RAG-/- hosts are REJECTED in WT Recipients

TIL cells (tumor infiltrating cells) include CTLs that recognize melanoma antigens/peptides (6/11 patients). But these CTLs were anergic: could not kill targets or produce γ-IFN.

- So...the good news is that immune recognition of tumor antigens occurs but the bad news is that this occurs without activation of immune effector responses.
Immune surveillance:
1. Innate system
   - NK, NKT, gamma/delta T cells
   - IFN-γ, IL-12 (APC)
2. Functional conventional T cells

More good news:
Evidence for Immunological Surveillance
- In mice, absence of IFN-γR, STAT1, IL-12, perforin, RAG, NK cells: All of these genetic deficiencies have an increased incidence of MCA (carcinogen) induced malignancies.
- Evidence that IFN-induced antigen presentation by tumor cells provides immunity (as with viral immunity). IFN-γR-/- tumors grow in WT mice, unless transfected with TAP. Highly immunogenic tumors emerge in RAG -/- mice; these tumors grow in RAG -/- (in absence of immune selective pressure) but are rejected in WT mice (in presence of normal immune response).
- Macrophages are primary sources of IL-12 which induce NK and T cell production of IFN-γ. (which in turn activates STAT1 in the tumor and in immune cells)

Immunization with Tumor Cells Can Induce Protective Immune Response

Tumor Antigens Are Unique to Individual Tumors

Candidate Tumor Antigens

Antigen Class | Antigen | Advances/Disadvantages
--- | --- | ---
Whole Cell | Protein lysate or tumor RNA based expression | "Customized" therapy are required for these approaches. For whole proteins "antigen profile" of each tumor is required. Peptides require additional info. of indiv. HLA-type Antigenic modulation or loss (overcome by attacking multiple targets and antigens required for transformed phenotype)
Antigen-Specific | Peptide, DNA or recombinant protein | (Autoimmunity may be a problem)
Candidate Tumor Antigens...many more to come through genomics

- **Shared Tumor Antigens** (common across tumors and tumor types) Allows single therapy to be applicable for many patients
  1. Cancer/testes genes
  2. Differentiation associated antigens
  3. Others including gangliosides, MUC-1, etc.
- **Unique Tumor Antigens** (requires tumor specific therapy) Antigenic modulation would potentially interfere with malignant phenotype.
  1. Overexpressed proto-oncogenes: EGFR, HER2
  2. Point mutations: ras, β-catenin, CDC27, CDK4, Bcr/Abl
  3. Viral Antigens: Human papilloma virus, EBV, Hepatitis B

### Antigen Class

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune-mediated</td>
<td>Thymoma, MM</td>
</tr>
<tr>
<td>T cell lymphomas</td>
<td>Colorectal, lung, bladder, thyroid and other cancer</td>
</tr>
<tr>
<td>Pancreatic, colon, lung, CML, ALL</td>
<td></td>
</tr>
<tr>
<td>Shared Tumor Antigens (common across tumors and tumor types)</td>
<td>Allows single therapy to be applicable for many patients</td>
</tr>
<tr>
<td>Tumor Specific Antigens</td>
<td>II, III, IV, V</td>
</tr>
<tr>
<td>Developmental Antigens (cancer/testes genes)</td>
<td>Seminoma, breast cancer</td>
</tr>
<tr>
<td>Human lymphoma virus (HLV)</td>
<td>Curved, purine cancer</td>
</tr>
<tr>
<td>Tumor-specific self-antigens (Differentiation antigens)</td>
<td>Tyrosinase, p105, pTyr-1, N-ras, and phosphatase, P53, Thymidylate synthase, retinoblastoma</td>
</tr>
<tr>
<td>Over-expressed self-antigens</td>
<td>MAGE-1, MAGE-3, GAGE family, 20 genes on the X chromosome</td>
</tr>
</tbody>
</table>

### Cross-presentation

- **Virus**
  - Exogenous pathway In draining LN
  - Immune activator-“danger” signals that induce T cell activation.
  - CD8
  - CD4

### Cross-Priming:

- **Induction of Anti-tumor T cell response**
  - Provide T(H)1 or 2 Help for B cell
  - Ab Responses
  - Class II + peptide
  - Endogenous presentation of peptide

### IMMUNE RECOGNITION

- **Tumor Evasion** Tumor cells are poorly immunogenic
  - Therefore cross-priming required (overcomes obstacles 1-4)

- **Host somatic cellular antigens (i.e not soluble antigens) are able to be presented to immune system by host APCs.**
- **True for viral antigens and cancer antigens.**

- **Phagocytosis**
- **Immature DC**
- **Activation ??**
- **Mature DC**

- **Antigen processing and presentation of antigen on class I and II**

- **APC**
- **T cell**
- **CD4**
- **CD8**
- **CD40**
- **CD40L**
- **CD28**
- **B7**
- **IL-2**
- **CTL**
- **ITAM**

- **TCR**
- **CD3**

- **MHC Class I/II**

- **Dendritic Cell**

- **NK cell**

- **Endogenous presentation of peptide**
- **Cross-Priming: Induction of Anti-tumor T cell response**

- **Therefore cross-priming required (overcomes obstacles 1-4)***

- **Post-APC**
  1. Thymocytes I
  2. Nonspecific
  3. Viral/lymphomatous antigens
  4. For adjuvant antibodies
  5. Overexpressed target cell
Tumor Immunity (Clynes)

**Effector Mechanisms**

**CD8 CTL Can Recognize Class I – peptide Complex and Induce Tumor Lysis and Apoptosis**

- Granule exocytosis: Perforin/granzyme
- Fas, FasL

**Effector Mechanisms**

**NK Cells Can Recognize Class I Negative Cells and Induce Tumor Lysis and Apoptosis**

- Granule exocytosis: Perforin/granzyme
- Fas, FasL

Yet, class I loss is common in cancer. Lack of activation of NK via activating NK receptors? Cytokine “milieu”?

**Effector Mechanisms**

**Macrophages are Cell-Mediated Effectors**

- TNF (+ other TNF-family members)
- NO, O2•, proteases

Cytokine-Mediated Activation

- IFN-γ
- GM-CSF
- TNF

**Effector Mechanisms**

**Antibody Bound Targets Induce Myeloid Cell Tumor Cytoxicity Through Fc Receptors +/- Complement Receptors**

- ADCC, phagocytosis, release of inflammatory mediators (NO, O2•, proteases, TNF, etc.,)

**Effector Mechanisms**

**FeR Mediated NK Cell – ADCC**

- ADCC

**Tumor Evasion of the Immune Response**

- Elimination
- Equilibrium
- Escape
Tumor Evasion: Two separate problems
• Tumor antigens are not recognized by immune response-poorly immunogenic (Immunologically ignorant).
• Tumors and tumor environment are resistant to or inhibit immune cytotoxic responses. (active suppression—either dampen “priming” or inhibit/resist effector cell function).

Bad News/Tumor Evasion Resistance to Effector Response
Tumor Cell Properties
• Immune resistance
  - Upregulation of genetic “survival” pathways (anti-apoptotic genes), e.g. akt, Bcl-2, etc.,
  - Resistance to death receptor pathways: Reduction of Fas receptor or enhanced expression of c-FLIP by tumors may render tumors resistance to fas-mediated apoptosis. Similarly, tumors commonly lose TRAIL receptors or express “decoy” receptors.
  - Loss of tumor antigen expression: Tumor heterogeneity (need to target multiple antigens) and possibly proteins essential for transformation/growth.
• Loss of antigen presentation capacity by tumor

More Bad News/Tumor Evasion Resistance to Effector Response
2 pages of problems… not good
Local Inhibitory Factors in the Tumor Stroma produced by tumor cells themselves or by stromal T cells or macrophages
• Tumor cell or Tumor-associated-macrophage production of local factors (TGF-β, IL-10) that suppress T cell responses and DCs (VEGF, and TGF-β, IL-10)
• Conventional T cells and DCs may be suppressed by Treg cells preferentially induced or recruited by tumor. (early clinical promise with Treg depleting approaches and anti-CTLA4 antibodies).
Autoimmunity Correlates With Tumor Regression in Patients With Metastatic Melanoma Treated With Anti-CTLA-4


**Alterations in Antigen Processing**

○ Proteosome, TAP loss, β2M loss, Class I loss or upregulation

○ Class I loss/regulated

○ Frequency (31-70%)

○ TAP/Proteosome(LMP7)-I0-80%

○ IFN-gammaR signaling defect

○ *related with metastatic and poor prognostic lesions

**Strategies for induction of anti-tumor Immune Responses**

- Passive-


  2. Monoclonal and engineered antibodies:

     1. Humanized/chimeric mAbs: Herceptin (anti-HER2), Rituxan (anti-CD20), anti-idiotypic (vaccine therapy), anti-EGFR (Erbitux), CAMPATH (anti-CD52), anti-VEGF (targets neovasculature, Avastin).

     2. Immune conjugates ("smart bombs") mAb-toxin (Mylotarg: anti-CD33 calicheamicin), mAb-chemo, mAb-isotope (anti-CD20 Zevalin and Bexxar).

**Therapeutic Antibodies in Oncology**

<table>
<thead>
<tr>
<th>mAb</th>
<th>Construct</th>
<th>Isotype</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituxin™ (anti-CD20)</td>
<td>Chimeric</td>
<td>IgG1</td>
<td>CD20</td>
</tr>
<tr>
<td>Herceptin™ (anti-HER2)</td>
<td>Humanized</td>
<td>IgG1</td>
<td>HER-2</td>
</tr>
<tr>
<td>Campath™ (anti-CD52)</td>
<td>Humanized</td>
<td>IgG1</td>
<td>CD20</td>
</tr>
<tr>
<td>Erbitux™ (anti-EGFR)</td>
<td>Chimeric</td>
<td>IgG1</td>
<td>EGFR</td>
</tr>
<tr>
<td>Avastin™ (anti-VEGF)</td>
<td>Humanized</td>
<td>IgG1</td>
<td>VEGF-A</td>
</tr>
<tr>
<td>Bexxar (Erlotinib+I727)</td>
<td>Mouse</td>
<td>IgG1</td>
<td>CD20</td>
</tr>
<tr>
<td>Zyt.mx™ (Bifunctional mAb)</td>
<td>Mouse</td>
<td>IgG1</td>
<td>CD20</td>
</tr>
<tr>
<td>Mylotarg™ (anti-CD33)</td>
<td>Humanized</td>
<td>IgG4</td>
<td>CD33</td>
</tr>
<tr>
<td>ABX-EGF</td>
<td>Human</td>
<td>IgG2</td>
<td>EGFR</td>
</tr>
</tbody>
</table>

**Potential Cytotoxic Mechanisms of Anti-Tumor Antibodies**

Potential mechanisms involve complement-mediated cytotoxicity, antibody-dependent cellular cytotoxicity, Fas-mediated apoptosis, MEK/ERK activation, and induction of pro-apoptotic caspase-3/-7.
**Fc Receptors Modulate Anti-Melanoma TA99 Monoclonal Antibody Efficacy**

<table>
<thead>
<tr>
<th>Normal Mouse</th>
<th>Mouse without Activating Fc receptors</th>
<th>Mouse without Inhibitory Fc receptors</th>
</tr>
</thead>
</table>

**Rituxan Clinical Responses in Follicular Lymphoma are Associated with High Affinity FcR Polymorphic Alleles**


**Strategies for induction of anti-tumor Immune Responses**

**ACTIVE IMMUNIZATION**

Goal is to define tumor antigens and then use them in an immunostimulatory fashion.

How to induce immune response and break tolerance: Essentially “the dirty little secret” of immunologists - the adjuvant effect; effective immunization usually requires mixing antigen with agents which promote uptake of antigen by APCs as well as activate and recruit APCs to vaccine site (e.g. Alum or Complete Freund’s Adjuvant: mineral oil/water emulsion + heat killed bacillus).

**How to present antigen: clinical trials**

- Systemic cytokines (e.g.IFNα): upregulate HLA/antigen processing, mature and activate APC
- Whole cell and adjuvant
- Tumor antigen protein or peptide and adjuvant
- Peptide and cytokines
- Turn cancer cell into an APC or a recruiter of APCs: transfect/infuse tumor with costim. gene (B7) or with cytokine gene (GM-CSF), DC tumor cell fusion.
- Gene gun (DNA vaccination:tumor specific gene+/−costimulatory+/−cytokine genes)
- Autologous DC’s “pulsed” with protein, peptides etc. Attempts to deliver tumor peptide for cytosolic class I loading in activated DCs.

**Tumor Immunology: Summary**

1) Immunological recognition of tumor occurs. Tumor specific T cells are found in patients, but are ineffective.
   - Classify the types of tumor antigens thus far identified.
2) Tumors have emerged in individuals having successfully overcome immunological surveillance.
   - Experimental data from mice are provided showing how immunological surveillance prevents tumor development and there are examples in humans in which cancers emerge in immunodeficient individuals.
3) Evasion mechanisms include reduced tumor antigen presentation and local immunoregulatory factors: inhibitory cytokines and cells.
4) Reversal of tolerogenic response is goal of immunotherapy
   - Passive immunization (anti-tumor antibodies, adoptive T cell therapy).
   - Active immunization (tumor-antigen plus adjuvant).
   - The goal is to induce antigen specific immunity while eliminating regulatory negative immunoregulatory signals.