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: (thyroiditis, hepatitis, pancreatitis (diabetes), vitiligo, ITP, AIHA, gradt 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.

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.) are not increased.
- 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 responses is implicated. (post-transplant patients are also at increased risk for melanoma and sarcoma).

Immunosurveillance: Tumors which Evolve in Lymphocyte Deficient Hosts are Rejected in WT Mice

Tumor (Sarcoma) Incidence is Increased in MCA-treated Lymphocyte Deficient Mice

Tumor Size
Host: RAG-/- WT

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

Tumor Incidence after MCA Treatment

0%
100%

IFNγ R-/-
WT

Tumor Expression of IFNγ Receptor is Required for Lymphocyte-Mediated Tumor Rejection

Transplanted tumor: IFNγR-/-

0%
100%

WT IFNγR-/- transfected with IFNγR

Tumor Size
Host: WT WT WT RAG-/-

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. Many patients make anti-tumor antibodies, but are mostly IgM-will not efficiently induce effector responses-and may indicate a lack of T cell priming.

- 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**

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#### 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 source of IL-12 which induce NK and T cell production of IFN-γ. (activates STAT1)

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#### Tumor Antigens Are Unique to Individual Tumors

**Immunized Tumor**

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
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<table>
<thead>
<tr>
<th>Tumor Challenge</th>
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<tr>
<td>Protection</td>
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**Candidate Tumor Antigens**

<table>
<thead>
<tr>
<th>Antigen Class</th>
<th>Antigen</th>
<th>Advantages/ Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Cell</td>
<td>Protein lyase or tumor RNA based expression</td>
<td>Universal (Autoimmunity may be a problem)</td>
</tr>
<tr>
<td>Antigen-Specific</td>
<td>Peptide, DNA or recombinant protein</td>
<td>“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).</td>
</tr>
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</table>
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

<table>
<thead>
<tr>
<th>Antigen Class</th>
<th>Antigen</th>
<th>Malignancy</th>
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<tbody>
<tr>
<td>Tumor Specific Antigen</td>
<td>Brmorgenibulin, Otxtype, ECR, M0t2ras, Mutant p53, p21-her2 abl fusion</td>
<td>Bladder, T cell lymphoma, Colorectal, lung, bladder, Head and neck cancer, Pancreatic, Colon, Lung, CML, ALL</td>
</tr>
<tr>
<td>Developmental Antigens</td>
<td>MAGE-I, MAGE-3, GAGE family, 20 genes on the Y chromosome Tumorant</td>
<td>Melanoma but also in colorectal, lung, gastric, Variance</td>
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<tr>
<td>(cancer/testes genes)</td>
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<tr>
<td>Viral Antigens</td>
<td>Human Papilloma Virus, EBV</td>
<td>Cervical, penile cancer, Burkitt’s lymphoma, nasopharyngeal Ca, post-Tx lymphoproliferative syndrome</td>
</tr>
<tr>
<td>Tissue-specific self-antigens</td>
<td>Tyrosinase, gp100-2np-1, nps-2, Prostatic acid phosphatase, PSA Thyroglobulin, sFas-Fragment</td>
<td>Melanoma, Prostate, Thyroid, Liver Cancer</td>
</tr>
<tr>
<td>(Differentiation antigens)</td>
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<tr>
<td>Over-expressed self-antigens</td>
<td>Her-2/neu, CEA, Mac-1</td>
<td>Breast and lung cancer, Colorectal, lung, breast, Colorectal, pancreatic, ovarian, lung</td>
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**Cross-presentation**

<table>
<thead>
<tr>
<th>Exogenous pathway</th>
<th>Immunity</th>
<th>Innate activator- “danger” signals</th>
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<tbody>
<tr>
<td>Virus</td>
<td>DC</td>
<td>CD8</td>
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**IMMUNE RECOGNITION**

**Cross-Priming**

- 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**

<table>
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<tr>
<th>Antigen processing and presentation of antigen on class I and II</th>
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<tbody>
<tr>
<td>Immature DC</td>
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</table>

**Maturation Factors**

- T cell signals (encounter with specific Memory CD4 cell): **CD40L**
- **Microbial stimuli**: TLR ligands: LPS, hypomethylated DNA (CpG), dsRNA (poly dI:dC), peptidoglycans,
- **Inflammatory Cytokines**: TNF, IFN, (products of either Mφ, NK or T cells)
**Effective antigen presentation by “cross-priming” enhanced by DC activation/maturation (CD40L, TNF, others)**

- Peripheral immature DCs migrate to LN upon activation by antigen/cytokines where they may encounter T cells.
- Maturation marked by transition of highly phagocytic/endocytic cell to a poorly phagocytic/endocytic cell.
- Upregulation of antigen processing and surface expression of class I and II molecules
- Upregulation of co-stimulatory molecules CD40, B7 (CD80,86) and adhesion molecules (ICAM-1) for interaction and activation of antigen-specific T cells.

**Effector Mechanisms**

- NK Cells Can Recognize Class I Negative Cells and Induce Tumor Lysis and Apoptosis
- Macrophages are Cell-Mediated Effectors
- CD8 CTL Can Recognize Class I –peptide Complex and Induce Tumor Lysis and Apoptosis
- Antibody Bound Targets Induce Myeloid Cell Tumor Cytotoxicity Through Fc Receptors +/- or Complement Receptors
**Tumor Evasion: Two separate problems**

- Tumor antigens are not recognized by immune response-poorly immunogenic (immunologically ignorant).
- Tumors are resistant to or inhibit immune cytotoxic responses.

  (active suppression—either dampen “priming” or avoid/inhibit/resist effector cell function).

**Bad News/Tumor Evasion**

**Resistance to Effector Response**

- Access to tumors may be limited by poor vascularity.
- Intrinsic resistance (anti-apoptotic genes).

Resistance to death receptor pathways: Reduction of Fas receptor or enhanced expression of c-FLIP by tumors may render tumors resistant to fas-mediated apoptosis.

Similarly, tumors commonly lose TRAIL receptors or express “decoy” receptors.

Upregulation of “survival” pathways…akt, Bel-2.

- Tumor cell or Tumor-associated-macrophage production of local factors (TGF-β, IL-10) that suppress T cell responses and DCs (VEGF, and TGF, IL-10)

**More Bad News/Tumor Evasion**

**Resistance to Effector Response**

- 2 pages of problems…not good
- Fasl expression on tumor cells may induce cell death of Fas + T cells.
- Conventional T cells may be suppressed by Treg cells preferentially induced or recruited by tumor.
- (early clinical promise with Treg depleting approaches and anti-CTLA4 antibodies).
- Antigen modulation (antibody-mediated endocytosis of surface antigen)
- 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
Tumor Immunity (Clynes)

Alterations in Antigen Processing

(Proteosome, TAP loss, β2M loss, Class I loss or upregulation)

Tumor Cell

Proteosome

TAP

β2M

Class I

Class I loss/reg’n

TAP/Proteosome

IFN-gammaR signaling defect (rare)

*associated with metastatic and poor prognostic lesions

Immunological Intervention: Early Successes

• Cooley’s toxin (gram + bacteria injected into tumor sites): local inflammatory rxn and systemic toxicity (fever, sepsis syndrome) associated with occasional tumor remissions (bacterial product induced production of IL-12, IFN-γ, TNFα – enhanced antigen presentation??)

• Systemic cytokines (IL-2, IL-12, IFN-α) 1980-90’s. Occasional responses (shrinkage in 5-15% of cases) with high toxicities. Higher responses for IFN-α in CML and hairy cell leukemia; CML remissions associated with anti-PR1 (proteinase in CML cells) T cell responses.

Strategies for induction of anti-tumor Immune Responses

- Passive -

• Adoptive transfer of T cells: Antigenic specific T cell clones requires HLA-restricted “customized” therapy or cytokine-enhanced antigen-non-specific T cells (LAK cells). Has worked for EBV lymphoproliferative disorders.

• Monoclonal and engineered antibodies:

  1. Humanized/chimeric mAbs: Herceptin (anti-HER2), Rituxan (anti-CD20), anti-idiotpe (custom therapy), anti-EGFR (Erbvax), 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).

Potential Cytotoxic Mechanisms of Anti-Tumor Antibodies

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).
Tumor Immunology: Summary

1) Immunological recognition of tumor occurs.

2) Tumors emerge in individuals having overcome immunological surveillance.

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 (antitumor antibodies, adoptive T cell therapy). Active immunization (vaccine=antigens plus adjuvant).

   The goal is to induce antigen specific effector T cells while eliminating regulatory negative immunoregulatory pathways.