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: (thyroiditis, 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.

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 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

Tumor:
WT origin  RAG-/- origin

Tumors which developed in RAG-/- hosts are REJECTED in WT Recipients
Tumor Immunity (Clynes)

**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.
- Highly immunogenic tumors emerge in RAG -/- mice spontaneously; these tumors grow in RAG -/- (in absence of immune selective pressure) but are rejected in WT mice (in presence of normal immune response).

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**Immunization with Tumor Cells Can Induce Protective Immune Response**

**Tumor Antigens Are Unique to Individual Tumors**

<table>
<thead>
<tr>
<th>Candidate Tumor Antigens</th>
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<tbody>
<tr>
<td>A</td>
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</table>

**Immunized Tumor**

<table>
<thead>
<tr>
<th>Tumor Challenge</th>
<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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<tbody>
<tr>
<td>Protection</td>
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<tr>
<td>No protection</td>
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</table>
**Tumor Immunity (Clynes)**

**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>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Specific Antigen</td>
<td>Immunoglobulin Idiotype</td>
<td>B lymphoma, MM</td>
</tr>
<tr>
<td></td>
<td>TCR</td>
<td>T cell lymphoma</td>
</tr>
<tr>
<td></td>
<td>Mutant ras</td>
<td>Colorectal, lung, bladder, Head and neck cancer</td>
</tr>
<tr>
<td></td>
<td>Mutant p53</td>
<td>Pancreatic, Colon, Lung</td>
</tr>
<tr>
<td></td>
<td>p21-/bcr-abl fusion</td>
<td>CML, ALL</td>
</tr>
</tbody>
</table>

**Developmental Antigens** (cancer/testes genes)

- MAGE-1, MAGE-3, GAGE family, 20 genes on the X chromosome
- Telomerase
- Melanoma but also in colorectal, lung, gastric

**Viral Antigens**

- Human Papilloma Virus
- EBV
- Cervical, penile cancer
- Burkitt's lymphoma, nasopharyngeal Ca, post-Tx lymphoproliferative

**Tissue-specific self-antigens** (Differentiation antigens)

- Tyrosinase, gp100, trp-1, trp-2
- Prostatic acid phosphatase, PSA
- Thyroglobulin
- α-Fetoprotein
- Melanoma
- Prostate
- Thyroid
- Liver Cancer

**Over-expressed self-antigens**

- Her-2/neu
- CEA
- Muc-1
- Breast and lung cancer
- Colorectal, lung, breast
- Colorectal, pancreatic, ovarian, lung

**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.

**DC Maturation**

- T cell signals (encounter with specific Memory CD4 cell): CD40L
- **Microbial stimuli**: TLR ligands: LPS, hypomethylated DNA (CpG), dsRNA (poly dI:dC), peptidoglycans, StAg,
- **Inflammatory Cytokines**: TNF, IFN, (products of either Mφ, NK or T cells)

**Maturation Factors**

- Poor APCs
  1) Often no class I
  2) No class II
  3) No costimulatory molecules
  4) Few adhesion molecules
  5) Antigenically largely self

Therefore cross-priming required (overcomes obstacles 1-4)
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:
  - Upregulation of antigen processing and surface expression of class I and II molecules (signal 1)
  - Upregulation of co-stimulatory molecules CD40, B7 (CD80,86), adhesion molecules (ICAM-1) and cytokines for interaction and activation of antigen-specific T cells (signal 2).

**Immune Recognition**

Cross-Priming: Induction of Anti-tumor T cell response

**Effector Mechanisms**

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

- Tumor Cell
- CD8 T
  - CTL
  - CD8
  - TCR
  - Class I
  - + peptide
  - IL-2
  - Provide T cell Help for B cell Ab Response

**Effector Mechanisms**

CD4 T Can Provide T cell Help for B cell Ab Responses

- CD4 T
  - TCR
  - CD4
  - + peptide
  - CD40
  - CD40L
  - IFN-γ
  - GM-CSF
  - TNF
  - TNF (and other TNF-family members)
  - NO, O2•, proteases

**Effector Mechanisms**

Macrophages are Cell-Mediated Effectors

- Macrophage
  - TCR
  - CD4
  - CD8
  - IFN-γ
  - GM-CSF
  - TNF
  - ADCC, phagocytosis, release of inflammatory mediators (NO, O2•, proteases, TNF, etc.)

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

**Alterations in Antigen Processing**

- Loss of function analogous to tumor suppressor loss -tumor progression?
- Proteosome, TAP loss, β2M loss, Class I loss or 4 upregulation
- Tumor Cell

**Bad News/Tumor Evasion**

**Resistance to Effector Response (Tumor Cell)**

- Loss of antigen presentation capacity by tumor
- 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 FLIP by tumors may render tumors resistance to fas-mediated apoptosis. Similarly, tumors commonly lose TRAIL receptors or express “decay” receptors.
- Upregulation of “survival” pathways...akt, Bcl-2
- Antigen modulation (antibody-mediated endocytosis of surface antigens)
- Loss of tumor antigen expression. Tumor heterogeneity (need to target multiple antigens) and possibly proteins essential for transformation/growth.

**More Bad News/Tumor Evasion**

**Resistance to Effector Response (Tumor Stroma)**

- 2 pages of problems...not good
- Tumor cell or Tumor-associated-macrophage production of local factors that suppress T cell responses (TGF-β, IL-10) and DCs (VEGF, and TGF, IL-10).
- Conventional T cells may be suppressed by Treg cells preferentially induced or recruited by tumor.
- *(early clinical promise with Treg depleting approaches and/or anti-CTL4 antibodies).*
Tumor Immunity (Clynes)

**FOXP3+ Tregs in human tumor (ascites), also in draining lymph node.**

**Tumor, DC and Mφ derived**

- A. "Immature"
- Producers of tolerogenic cytokines (IL-10, TGF-β) & IDO (effects tryptophan metabolism)

**B. Tolerogenic DC inhibit effector T cell activation**

**Cancer and Inflammation: Seed and Soil Hypothesis**

"Stromal inflammation as tumor "promoter" --"tolerogenic" healing/remodeling/repair

- Seed: Inflammatory stimulus
- Soil: Stromal inflammation as tumor "promoter"

<table>
<thead>
<tr>
<th>Malignancy</th>
<th>Inflammatory stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder cancer</td>
<td>Schistosomiasis</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>H. pylori-induced gastritis</td>
</tr>
<tr>
<td>MALT lymphoma</td>
<td>H. pylori</td>
</tr>
<tr>
<td>Hepatocellular CA</td>
<td>Hepatitis virus (B and C)</td>
</tr>
<tr>
<td>Kaposi's sarcoma</td>
<td>HHV8</td>
</tr>
<tr>
<td>Lung CA</td>
<td>Silica, Asbestos</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>Asbestos</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Salpingitis/taut/violation/endometriosis</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>Barrett's metaplasia</td>
</tr>
<tr>
<td>Papillary thyroid CA</td>
<td>Thyroiditis</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Prostatitis</td>
</tr>
</tbody>
</table>

**Tumor microenvironment**

- IL-6
- CD11b +/Gr-1 + monocytes
- iNOS +/Arg +
- Highly reactive peroxynitrite: can modify tyrosines
  - Induce T cell anergy/apoptosis
  - Chemically modify TCR

**Tumor Associated Macrophages/ Myeloid Suppressor Cells**

- RANTES
- MIP-1α, MCP-1, TNF-α
- Arginase, iNOS
- Inflammatory stimulus

**Macrophage Products that Drive Tumorogenesis**

- Growth and survival
  - Basic FGF, EGF, hepatocyte growth factor, PDGF, IL-6, TNF, polyamines, PGE2
- Angiogenesis
  - VEGF, MMP-9, IL-1β, IL-8, insulin-like growth factor (IGF-I), IGF-II, PGE2
- Tissue invasion and metastases
  - Chemokines, PGE2, matrix metalloproteinases, uPA, plasmin
- Mutations
  - Superoxide, peroxynitrite
- Inhibition of T cell responses
  - IL-10, TGF-β, indoleamine-2,3-dioxygenase, PGE2, superoxide, peroxynitrite, arginase

**On the near horizon: Removing immuno-inhibitory pathways**

- **Anti-CTLA4 Abs**: 15% clinical response in melanoma, prostate, etc., Autoimmunity seen in many patients. Combined therapy with tumor vaccines ongoing.
- **Treg depletion** (IL-2 Diptheria toxin conjugate)
- **Anti-PD-1**: Reversal of T cell exhaustion?
Tumor Immunity (Clynes)

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

- **Passive**
  - **Adoptive transfer of T cells**: Antigen-specific T cell clones requires HLA-restricted "customized" therapy or cytokine-enhanced antigen-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-idiotypic (custom therapy), anti-EGFR (Erbibux), CAMPATH (anti-CD52), anti-VEGF (targets neovascularature, Avastin).
  2. Immune conjugates ("smart bombs"):
     - tumor-targeted antibodies can deliver toxic payloads.
     - mAb-toxin (Mylotarg: anti-CD33 calicheamicin), mAb-chemo, mAb-isotope (anti-CD20 Zevalin and Bexxar).

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**Antibody Therapy in Cancer**

- Chemo (CHOP) + anti-CD20 mAb
- Chemo (CHOP) alone

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

- Complement-mediated lysis of target cells
- Killing of macrophages or natural killer cells
- Inducing tumor necrosis or growth arrest

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**Fc Receptors Modulate Anti-Melanoma TA99 Monoclonal Antibody Efficacy**

- WT vs. Ab
- More effective in the presence of FcγR

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**Passive Adoptive T Cell Immunotherapy in Metastatic Melanoma**

- Autologous TIL Cells + IL-2
- Infuse 10 billion cells
- Lymphodepleted patients
- Persistent expansion of tumor-specific T cells in patients
- 18/35 objective responses and 4 complete responses.
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 both
promote uptake of antigen by APCs as well as activate and recruit APCs to vaccine site (e.g. classic adjuvants:
Alum or Complete Freund’s Adjuvant: mineral oil/water emulsion + heat killed bacillus; molecular adjuvants: TLR
ligands, CD40L).

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/infect 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.
2) Tumors emerge in individuals having successfully overcome immunological surveillance.
3) Evasion mechanisms include reduced tumor antigen presentation
   and local immunoregulatory factors: inhibitory cytokines and cells.
4) Tumor development may both be promoted by chronic inflammation
   and be sustained by the tolerogenic tumor stroma microenvironment.
5) Reversal of tolerogenic response is the goal of immunotherapy
   Passive immunization (antitumor antibodies, adoptive T cell therapy).
   Active immunization (vaccine–antigen plus adjuvant).
   The goal is to induce antigen specific effector T cells while eliminating
   negative immunoregulatory effectors.