Tumor Immunity (Clynes)

Cross-presentation

Virus

Tolerance

Exogenous pathway

In draining LN

Immunity

Innate activator-
“danger” signals

CD8

DC

CD4

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.

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.

More good news/
Evidence for Immunological Surveillance

Humans

- 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 Incidence

0%

100%

RAG-/- WT

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

Tumor Size

Host: RAG-/- WT

Tumor: WT origin

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

Tumor: RAG-/- origin

Where is the danger—the innate activator?
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)
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>&quot;Customized&quot; 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>
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Advantages/ Disadvantages of Candidate Tumor Antigens

- **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

Candidate Tumor Antigens..many more to come through genomics

<table>
<thead>
<tr>
<th>Antigen Class</th>
<th>Antigen</th>
<th>Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Specific Antigen</td>
<td>Stromelysin, Tumor Inhibitory Factor</td>
<td>B lymphomas, SS</td>
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<tr>
<td></td>
<td>TGF-α</td>
<td>T cell lymphomas</td>
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<tr>
<td></td>
<td>Traf2, ras</td>
<td>Colorectal, lung, bladder</td>
</tr>
<tr>
<td></td>
<td>Mutant p53, p21</td>
<td>Head and neck cancer</td>
</tr>
<tr>
<td>Developmental Antigens (cancer/testes genes)</td>
<td>MAGE-1, MAGE-3, GAGE family</td>
<td>Melanoma but also in colorectal, lung, prostate</td>
</tr>
<tr>
<td></td>
<td>20 genes on the Y chromosome</td>
<td>Various</td>
</tr>
<tr>
<td>Viral Antigens</td>
<td>Human Papilloma Virus</td>
<td>Cervical, penile cancer</td>
</tr>
<tr>
<td></td>
<td>EBV</td>
<td>Burkitt’s lymphoma, nasopharyngeal Ca, post- Tx lymphoproliferative</td>
</tr>
<tr>
<td>Tissue-specific self-antigens (Differentiation antigens)</td>
<td>Tyrosinase, gp100, gp-1, my-2</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Prostatic acid phosphatase, PSA</td>
<td>Prostate</td>
</tr>
<tr>
<td></td>
<td>Thyroglobulin</td>
<td>Thyroid</td>
</tr>
<tr>
<td></td>
<td>α-Fetoprotein</td>
<td>Liver Cancer</td>
</tr>
<tr>
<td>Over-expressed self-antigens</td>
<td>Her-2/neu</td>
<td>Breast and lung cancer</td>
</tr>
<tr>
<td></td>
<td>CEA</td>
<td>Colorectal, lung, breast</td>
</tr>
<tr>
<td></td>
<td>Mac-1</td>
<td>Colorectal, pancreatic, ovarian, lung</td>
</tr>
</tbody>
</table>

Tumor Evasion
Tumor cells are poorly immunogeneic

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

Poor APCs
- Poor MHC Class I
- No class II
- No costimulatory molecules
- No antigenically large self

IMMUNE RECOGNITION

Cross-Primming

- 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

Dendritic Cell

Immature DC

Antigenic processing and presentation of antigen on class I and II

Activation ?? Mature DC

DC Maturation

IMMUNE RECOGNITION

Tumor Evasion
Tumor cells are poorly immunogeneic

Therefore cross-priming required (overcomes obstacles 1-4)
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, StAg,
- 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 cytokines, chemokines, costimulatory molecules CD40, B7 (CD80,86) and adhesion molecules (ICAM-1) for interaction and activation of antigen-specific T cells.
Tumor Immunity (Clynes)

**Effecter Mechanisms**

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

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

**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 resistance to fas-mediated apoptosis.
- Similarly, tumors commonly lose TRAIL receptors or express “decoy” receptors.
- Upregulaton 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 or by CTLA4 (early clinical promise with CTLA4lg).
- 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

Alterations in Antigen Processing

(Loss of function analogous to tumor suppressor loss -tumor progression?)

- Proteosome, TAP loss, β2M loss, Class I loss or upregulation
- Tumor Cell

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-idiotypic (custom therapy), anti-EGFR (Erbitux), CAMPATH (anti-CD52), anti-VEGF (targets neovascularature, 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

Monoclonal Antibody Therapeutics in Cancer

Rituxan (anti-CD20)
- High response rate in B cell lymphoma (>70%).
- Synergy with chemotherapy or XRT.
- Recognizes B cell marker regulating B cell activation.
- Induces growth arrest/apoptosis in vitro.

Herceptin (anti-HER2)
- Lower response rate in breast cancer (15%).
- Synergy with chemo (60%) or XRT.
- Recognizes EGF-like receptor regulating cellular proliferation (ERBB2).
- Induces growth arrest/apoptosis in vitro.

Monoclonal Anti-tumor Antibody Approaches in Cancer

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 Immunity (Clynes)

**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: transfec/infec 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.

**Manipulation of DCs for Immunotherapy**

- Autologous DC’s “loaded” with
  - **Peptides of tumor antigens:** (early 10-30% partial response rate in advanced prostate CA and melanoma) practical problems: lack of knowledge of 1) tumor antigens 2) HLA-restricted (available only for the most common HLA-types, 3) antigen modulation most likely results in evasion for a small # of epitopes)
  - **Known recombinant tumor antigens (whole protein)**
    - Idiotype for B cell lymphoma works but laborious
  - **Antigen non-specific approaches:** Tumor lysates, Apoptotic bodies, RNA encoding known tumor antigens, RNA derived using subtraction libraries, DNA encoding known tumor antigens, Tumor-DC fusions
- **DC delivery into tumors**
  - Mobilization using Flt-3