Immunotherapeutic barriers at the level of the tumor microenvironment

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CD8+ cytotoxic T lymphocyte killing an antigen-expressing tumor cell
In vivo, a tumor is more than tumor cells

- Three dimensional mass
- Extracellular matrix
- Supported by the neovasculature, fibroblasts, macrophages
- Variable presence of inflammatory cells
  - T cells (and subsets thereof)
  - B cells/plasma cells
  - NK/NKT cells
  - Dendritic cell subsets
- The functional phenotypes of these cells may or may not be permissive for an effective anti-tumor immune response (either priming phase or effector phase)
- Also, likely need for dynamic interaction with draining lymph node compartment for optimal anti-tumor immunity ➔ added complexity
Complexity of stromal elements in solid tumors

DeMorrow et al. 2011
Anti-tumor immune responses in vivo: Taking into account the tumor microenvironment

**Lymph node (Priming phase)**

- APC
  - nCD8
  - IL-2
  - eCD8

- Adoptive T cell transfer

**Tumor microenvironment (Effector phase)**

- APC
  - eCD8

- Chemokines
  - IFN-γ

- Granzymes, perforin

- Inhibitory mechanisms

- Lymphatic

- Blood

**Vaccine**

**Endogenous**
Features of subsets of solid tumors that might mediate poor immune recognition or lack of immune destruction

- Priming phase
  - Lack of innate immune-activating “danger” signals
  - Poor recruitment of the critical APC subsets for cross-presentation of antigens to T cells
  - Inadequate expression of costimulatory ligands on tumor cells or on infiltrating APCs

- Effector phase
  - Inadequate recruitment of activated effector T cells
    - Vascular endothelial cells/homing receptors
    - Chemokines
  - Presence of dominant immune inhibitory mechanisms that suppress T cell effector functions
    - Inhibitory receptors (e.g. PD-L1/PD-1)
    - Extrinsic suppressive cells (e.g. Tregs, MDSCs)
    - Metabolic inhibitors (e.g. IDO, arginase)
    - Inhibitory cytokines (e.g. IL-10, TGF-β)
Hypothesis

• Features of the tumor microenvironment could dominate at the effector phase of the anti-tumor T cell response and limit efficacy of current immunotherapies
  – T cell trafficking into tumor
  – Immune suppressive mechanisms at tumor site
  – Tumor cell biology and susceptibility to immune-mediated killing
  – Complexities of the tumor stroma (vasculature, fibrosis)
• Reasoned that these features could be interrogated through pre-treatment gene expression profiling of tumor site in each individual patient
• Such an analysis could identify a predictive biomarker profile associated with clinical response, and also highlight new biologic barriers that need to be overcome to optimize therapeutic efficacy of vaccines and other immunotherapies
Expression of a subset of chemokine genes is associated with presence of CD8$^+$ T cells in melanoma metastases

Patients with clinical benefit from immunotherapies

Harlin et al.
Gene expression pattern of tumor microenvironment associated with favorable clinical outcome to a dendritic cell vaccine

Schuler collaboration, ASCO 2009
Chemokine/T cell gene expression signature is associated with survival following GSK MAGE3 protein vaccine

Louahed et al., EORTC-NCI-AACR 2009
Ipilimumab clinical responders also show a chemokine/T cell gene expression profile in tumor microenvironment.

- CXCL9, 10, 11
- CCL4, CCL5
- Granzyme B
- Perforin
- CD8α

Ji et al, AACR 2011
Implication of melanoma gene array results for patient-specific therapy

- Gene expression profiling of the melanoma tumor microenvironment has revealed reproducible patterns associated with clinical benefit ➔ should be explored as predictive biomarker in prospective trials
  - Already being pursued by GSK-Bio in context of multicenter MAGE3 vaccine studies

- Ideally, this strategy should allow enrichment for the potentially responsive patient population in the future
  - Think Her2 equivalent for T cell immunotherapies

- These observations also highlight critical aspects of tumor/immune system biology, and suggest specific strategies for overcoming immunologic barriers at the level of the tumor microenvironment
Two broad categories of tumor microenvironments defined by gene expression profiling and confirmatory assays

- **T cell “rich”**
  - Chemokines for T cell recruitment
  - CD8+ T cells in tumor microenvironment
  - Broad inflammatory signature
  - Apparently predictive of clinical benefit to vaccines

- **T cell “poor”**
  - Lack chemokines for recruitment
  - Low indicators of inflammation

What dictates recruitment of activated CD8+ T cells into tumor sites?
What are the innate immune mechanisms that promote T cell priming in a subset of patients?
Why are tumors that contain activated CD8+ T cells not rejected spontaneously?

Gajewski, Brichard; Cancer J. 2010
1. Chemokines, vascular endothelium, and T cell migration into tumor sites

What is attracting T cells into some tumors? Can we mimic this in the tumors that fail to achieve it spontaneously?
A subset of melanoma cell lines expresses a broad array of chemokines

- Implies that in some cases, the melanoma tumor cells themselves can produce a broad panel of key chemokines for T cell migration
Superior recruitment of human CD8+ effector T cells in NOD/scid mice bearing “chemokine-high” M537 melanomas

Harlin et al.
Features of vascular endothelial cells also regulate T cell homing: ET$_B$R

Candidate strategies to promote effector T cell migration into tumor sites

- Introduce chemokines directly
  - CXCR3-binding chemokines (CXCL9, CXCL10)
  - Others (CCL2, CCL3, CCL4, CCL5)
- Induce chemokine production from stromal cells
  - LIGHT, lymphotoxin: bind LTβR
- Elicit appropriate local inflammation that includes chemokine production
  - Type I IFNs
  - TLR agonists
  - Radiation
- Alter signaling pathways in melanoma cells themselves to enable chemokine gene expression by tumor cells
Intratumoral LIGHT adenovirus in B16 melanoma: Promotes chemokine production, CD8+ T cell recruitment, primary tumor control, and rejection of non-injected distant metastases

Yu et al, J. Immunol. 2007
2. T cell suppressive mechanisms

Why are TIL not eliminating the tumor cells they are infiltrating? Can we overcome this defect and restore tumor rejection?
Inflamed melanomas containing CD8\(^+\) T cells have highest expression of immune inhibitory pathways

- **IDO** (indoleamine-2,3-dioxygenase)
  - Tryptophan depletion
- **PD-L1**
  - Engages PD-1 on T cells
- **CD4\(^+\)CD25\(^+\)FoxP3\(^+\)Tregs**
  - Extrinsic suppression
- **T cell anergy** (B7-poor)
  - T cell intrinsic TCR signaling defect

Correlated expression of IDO, FoxP3, and PD-L1 transcripts in individual tumors
Interfering with PD-L1/PD-1 interactions can lead to tumor rejection in vivo

Blank et al, Cancer Research, 2004
1-methyltryptophan reverses immunosuppression by IDO and enables tumor control in vivo

*Uyttenhove et al Nature Med. 9:1269, 2003*
Uncoupling multiple immune suppressive mechanisms in combination: Treg depletion and anergy reversal synergize to promote rejection of B16 melanoma and vitiligo

Strategies to block immune inhibitory mechanisms tested in mouse models and being translated to the clinic

• **IDO inhibition**
  – 1-methyltryptophan (RAID program)
  – New more potent IDO inhibitors (Incyte)

• **Blockade of PD-L1/PD-1 interactions**
  – Anti-PD-1 and anti-PD-L1 mAbs (BMS, Merck, Curetech, Genentech)

• **Depletion of CD4+CD25+FoxP3+ Tregs**
  – Ontak (IL-2/DT fusion)
  – Daclizumab, Basiliximab (anti-IL-2R mAbs)
  – Ex vivo bead depletion of CD25+ cells from T cell product for adoptive transfer

• **Anergy reversal**
  – Introduction of B7-1 into tumor sites
  – Homeostatic cytokine-driven proliferation
    • T cell adoptive transfer into lymphopenic recipient
    • Exogenous IL-7, IL-15

• **Combinations of negative regulatory pathway blockade**
  – Synergy between blockade of 2 or more pathways
Anti-PD-1 mAb phase I (MDX-1106; BMS 936558): Tumor response

Responses also seen in NSCLC and renal cell carcinoma;
Topalian update 2012: 27% RR among 95 melanoma patients
Reduction of Treg number using Denileukin diftitox can have clinical activity in melanoma

Rasku et al

Multicenter phase II study currently ongoing
Dose-dependent inhibition of IDO activity as assessed by kynurenine/tryptophan ratios in treated patients

Newton et al. ASCO 2012
2b. Solid tumor stroma as a barrier

How do stromal components that support tumor growth interface with host immune response?
Targeting tumor stroma immunologically may be the key to durable complete responses

Anti-CD40 mAb promotes tumor shrinkage by altering intratumoral macrophages in pancreatic cancer

Beatty, Vonderheide et al. Science 2011
3. Innate immune sensing of tumor—type I IFNs

How are anti-tumor T cells sometimes becoming spontaneously primed? Can we improve endogenous T cell priming in the tumors that fail to do so alone?
Melanoma metastases that contain T cell transcripts also contain transcripts known to be induced by type I IFNs

A: IRF1

B: IFN-induced p30
Host IFN-$\alpha/\beta$R is critical for generating a spontaneous tumor-specific T cell response

A: IFN-$\alpha/\beta$Rko

B: Stat1ko
Mice deficient in IFN signaling fail to accumulate CD8α+ DC subset in tumor microenvironment

A: Percentage

B: Absolute number
Batf3\(^{-/-}\) mice (deficient in CD8\(\alpha\) DCs) fail to spontaneously prime anti-tumor T cells, downstream from IFN-\(\beta\)

A: ELISPOT

B: Tetramer

C: IFN-\(\beta\) production
Type I IFN signaling must occur on the Batf3-dependent cell subset in order to support spontaneous CD8$^+$ T cell priming to tumor

*Fuertes et al; J. Exp. Med. 2011*
Innate immune sensing of tumors drives host type I IFN production and cross-priming of CD8+ T cells via CD8α DCs.
Provision of exogenous IFN-\(\beta\) intratumorally can potently induce tumor rejection
Conclusions

• There is heterogeneity in patient outcome to immune-based therapies for cancer such as melanoma vaccines, IL-2, and anti-CTLA-4 mAb
• One component of that heterogeneity is derived from differences at the level of the tumor microenvironment
• Key determining factors in melanoma microenvironment include chemokine-mediated recruitment of effector CD8⁺ T cells, local immune suppressive mechanisms, and innate immune activation including type I IFNs
• Understanding these aspects is enabling improved patient selection for Rx with immunotherapies (predictive biomarker), and also development of new interventions to modify the microenvironment to better support T cell-mediated rejection
• Targeting the tumor stroma immunologically may be just as critical as targeting the tumor cells
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Costa Rica 2012