Regulation of anti-tumor immunity through migration of immune cell subsets within the tumor microenvironment

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Founding 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
Anti-tumor immune responses: Taking into account the effector phase in the tumor microenvironment

Lymph node (Primming phase)

- APC
- nCD8
- IL-2

Blood

- eCD8

Tumor microenvironment (Effector phase)

- APC
- Chemokines
- IFN-γ
- Granzymes
- perforin
- Inhibitory mechanisms

Vaccine

Endogenous

Lymphatic
Affymetrix gene array analysis of pre-treatment biopsies from patients on melanoma vaccine sorted by clinical outcome

6 months SD or better: Includes transcripts for TCRα, CXCL9, CCL21
Expression of a subset of chemokine genes is associated with presence of CD8 transcripts

CD8β
CCL2
CCL4
CCL5
CXCL9
CXCL10
CCL19
CCL21
A subset of melanoma cell lines expresses a broad array of chemokines

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

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<th>Blood</th>
<th>Spleen</th>
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<td><strong>M537 tumor carrier</strong> (“Chemokine-high”)</td>
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<td><strong>M888 tumor carrier</strong> (“Chemokine-low”)</td>
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Chemokine-mediated recruitment of CD8⁺ effector T cells into tumor microenvironment

• Effector CD8⁺ T cells upregulate expression of CCR1, CCR2, CCR5, and CXCR3
• Migration supported by 6 chemokines that act via these receptors: CCL2, -3, -4, -5 and CXCL9, -10
• A subset of melanoma cell lines expresses a range of chemokines capable of recruiting CD8⁺ effector cells in vitro and in vivo
• Experiments aimed at determining the most critical of these chemokines to express in the tumor microenvironment to promote optimal recruitment are ongoing

Harlin et al.  
Two broad categories of tumor microenvironments defined by gene expression profiling and confirmatory assays

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

- **T cell “rich”**
  - Chemokines for T cell recruitment
  - CD8⁺ T cells in tumor microenvironment
  - Broad inflammatory signature
  - Apparently predictive of clinical benefit to several vaccines
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Why are tumors that contain activated CD8+ T cells not rejected spontaneously?

Gajewski, Brichard Cancer J. 2010
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What are the innate immune mechanisms that promote spontaneous T cell priming in a subset of patients?

Gajewski, Brichard Cancer J. 2010
Why are melanomas that do attract CD8+ T cell not rejected spontaneously?

- **IDO** (indoleamine-2,3-dioxygenase)
- **PD-L1** (engages PD-1)
- **CD4+CD25+FoxP3+Tregs**
- T cell **anergy** (B7-poor)

IHC for IDO, FoxP3, and PD-L1 shows expression in distinct cell subsets in melanoma metastases
Correlated expression of IDO, FoxP3, and PD-L1 transcripts in individual tumors

Note: these are highest in tumors that contain CD8\(^+\) T cells
Is there a causal relationship between the accumulation of CD8⁺ T cells and the presence of immune inhibitory pathways?

- It is assumed that the tumor establishes an immune suppressive microenvironment so that T cells that infiltrate become inhibited.
- However, we observe higher expression of immune inhibitory pathways in tumors that contain T cells.
- New hypothesis:
  - The expression of IDO and PD-L1, and the accumulation of Tregs, may depend on the infiltration of CD8⁺ T cells in the tumor site.
  - These might be induced by specific factors produced by activated CD8⁺ T cells.
- To test these notions, in vivo mouse models were utilized.
Supernatant from activated human CD8\(^+\) T cells recruits sorted CD4\(^+\)CD25\(^+\) T cells in a CCL22-dependent fashion
Superior migration of human Tregs into melanoma xenograft when CD8s are co-transferred
Summary of regulation of immune suppressive mechanisms in the tumor microenvironment

• The three major immune inhibitory mechanisms confirmed to be present in the melanoma tumor microenvironment appear to be immune-intrinsic, driven by CD8$^+$ T cells and not driven by the tumor
• For IDO and PD-L1, IFN-$\gamma$ is the major mediator
• For Tregs, CCL22 production by CD8$^+$ effector cells is the major mediator
• Blockade of these mechanisms therefore represent attractive strategies to restore anti-tumor T cell function and promote tumor rejection in patients
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 (Medarex/BMS)

- **Depletion of CD4⁺CD25⁺FoxP3⁺ Tregs**
  - Ontak (IL-2/DT fusion)
  - Daclizumab (anti-IL-2R mAb)
  - 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
  - Decipher molecular mechanism and develop small molecule inhibitors to restore T cell function

- **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

~30% response rate also seen in NSCLC and renal cell carcinoma

Sznol et al. ASCO 2010
What initiates spontaneous T cell priming and recruitment in a subset of melanomas?

Melanoma metastases that contain T cell transcripts also contain transcripts known to be induced by type I IFNs

**A: IRF1**

**B: IFN-induced p30**
Implantation of B16 melanoma results in IFN-β production in the tumor-draining lymph node
Multiple tumor types elicit IFN-β production in the tumor-draining lymph node

A: IFN-β mRNA in DLN cells

B: IFN-β mRNA based on CD11c
Host IFN-α/βR is critical for generating a spontaneous tumor-specific T cell response

A: IFN-α/βRko

B: Stat1ko
New questions surrounding IFN-β-centered innate immune response to tumors

1. What are the tumor-derived factors that induce IFN-β on host CD11c⁺ cells, and through what receptor system?

2. By what mechanism is IFN-β promoting cross-priming of host CD8⁺ T cells by DCs?
What are the APC defects in type I IFNRko (or Stat1ko) mice?

- Bone marrow chimera and adoptive transfer experiments map the defect to the level of host APCs.
- Apparently normal:
  - Numbers of dendritic cell subsets in spleen and tumor-draining LNs (mDC, CD8α DC, pDC)
  - Expression of MHCI/II, CD40, B7-1, B7-2 by these DC subsets
  - Ability of DCs to stimulate naïve CD8+ TCR Tg T cells in vitro
  - Migration of DCs from skin to lymph node (FITC painting)
  - Expression of class I/SIY peptide complexes (using TCR tetramer) on intratumoral APCs (CD11b+, CD11c+)
Anti-tumor immune responses: Working model for innate immune signals and tumor antigen cross-presentation
Conclusions

- Multiple key factors in the tumor microenvironment linked to immune-mediated tumor control depend on regulated recruitment of inflammatory cell subsets.
- This includes the priming phase (CD8α+ DC recruitment), the effector phase (CD8+ effector cell recruitment) and negative regulation (Treg recruitment).
- CD8+ effector T cells appear to be recruited via CCL2-5 and/or CXCL9-10, whereas Tregs are largely recruited via CCL22 produced by activated CD8+ T cells.
- Innate immune recognition of tumor, when it does occur, drives expression of Type I IFNs.
- Type I IFN signals drive recruitment of the CD8α+ DC subset--participating chemokine(s) currently being evaluated.
- Understanding these aspects should enable the development of new interventions to modify the microenvironment and better support T cell-mediated rejection.
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