Innate and adaptive immunity regulated from within the tumor microenvironment

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Expression of a subset of chemokine genes is associated with presence of CD8$^+$ T cells in melanoma metastases

Patients with clinical benefit from immunotherapies

Chemokine/T cell gene expression signature is associated with survival following GSK MAGE3 protein vaccine

AS15: HR (GS+ vs GS-) = 0.268 (95%CI [0.08;0.90])
AS02B: HR (GS+ vs GS-) = 0.433 (95%CI [0.17;1.14])

Louahed et al., EORTC-NCI-AACR 2009
Two broad categories of melanoma metastases 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 several immunotherapies

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

What molecular mechanisms explain these two phenotypes?

What are the innate immune mechanisms that promote spontaneous T cell priming in some patients?

Gajewski, Brichard; Cancer J. 2010
1. Hypothetical mechanisms that could explain spontaneous T cell-based inflammation in tumor microenvironment in a subset of patients

A. Somatic differences at the level of tumor cells
   – Oncogene pathways differentially activated
   – Mutational landscape

B. Germline genetic differences at the level of the host
   – Polymorphisms in immune regulatory genes

C. Environmental differences
   – Intestinal microbiome
   – Immunologic exposure history of patients
A. T cell infiltrate in mouse melanoma can be excluded by expression of accessory oncogene in a genetic tumor model

Oncogene combinations that give melanomas with Tyr-CreER and topical 4-HO Tam:

- Braf V600E + oncogene X
- Braf V600E + oncogene Y
- Braf V600E + oncogene X + oncogene Y

Statistical significance:
- Braf V600E + oncogene X vs. Braf V600E + oncogene Y: p = 0.0003
- Braf V600E + oncogene X vs. Braf V600E + oncogene Y + oncogene Y: p = 0.0003
- Braf V600E + oncogene Y vs. Braf V600E + oncogene Y + oncogene Y: n.s.
B. Loss of inflamed gene expression pattern in B16 tumors grown in type I IFNR−/− mice

- Diminished expression of chemokines and T cell markers that recapitulates human subsets
- Implies that genetic variability in type I IFN pathway is one hypothetical mechanism that could explain differential immune phenotypes

Selected transcripts downregulated:

<table>
<thead>
<tr>
<th>IFN-induced genes</th>
<th>Chemokines</th>
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<tbody>
<tr>
<td>IFI27</td>
<td>CCL2</td>
</tr>
<tr>
<td>IFI44</td>
<td>CCL5</td>
</tr>
<tr>
<td>IFI202B</td>
<td>CXCL9</td>
</tr>
<tr>
<td>IFI203</td>
<td>CXCL10</td>
</tr>
<tr>
<td>IFI35</td>
<td>CXCL13</td>
</tr>
<tr>
<td>IFN-induced p30</td>
<td></td>
</tr>
<tr>
<td>IRF7</td>
<td></td>
</tr>
</tbody>
</table>

**T cell markers**

<table>
<thead>
<tr>
<th></th>
<th>Other immune genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCRα</td>
<td>CD40</td>
</tr>
<tr>
<td>TCRβ</td>
<td>CD83</td>
</tr>
<tr>
<td>CD3γ</td>
<td>CD86</td>
</tr>
<tr>
<td>Itk</td>
<td>FcγR1</td>
</tr>
<tr>
<td>Fyb</td>
<td>Complement C1q</td>
</tr>
<tr>
<td>Granzyme B</td>
<td>Complement factor B</td>
</tr>
<tr>
<td>Perforin</td>
<td>IL-18</td>
</tr>
<tr>
<td>CD28</td>
<td>CD68</td>
</tr>
<tr>
<td></td>
<td>Class II MHC</td>
</tr>
</tbody>
</table>
2. Innate immune signals—
type I IFNs and tumor sensing

How are anti-tumor T cells sometimes becoming spontaneously primed? What is the innate immune sensing mechanism that drives adaptive immunity against tumors?
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**
Innate immune sensing of tumors drives host type I IFN production and cross-priming of CD8+ T cells via CD8α DCs

Fuertes et al; J. Exp. Med. 2011
Innate sensing toward IFN-β production
Tumor-derived DNA induces IFN-β production from DCs in an IRF3- and STING-dependent fashion

A: Tumor-derived preparations

B: IRF3 and STING-/-

DNA includes lipofectamine; RNA not effective
In vivo verification:
Host STING and IRF3 are required for spontaneous induction of CD8$^+$ T cell responses against tumor-derived antigen in vivo

A: STING$^{-/-}$

B: IRF3$^{-/-}$
Rejection of immunogenic tumors is ablated in STING^-/- mice

B16.SIY melanoma in 129 mice

Similar results in 3 immunogenic mouse tumor models
Model for tumor-induced DC activation and subsequent T cell priming

Woo et al; manuscript in preparation
3. T cell suppressive mechanisms

Why are TIL not eliminating the tumor cells they are infiltrating? Can we overcome this defect and restore tumor rejection?
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)

Correlated expression of IDO, FoxP3, and PD-L1 transcripts in individual tumors

Note: these are highest in tumors that contain CD8\(^+\) T cells
Presence of Tregs and expression of PD-L1 and IDO are associated with a CD8$^+$ T cell infiltrate

Correlations also between CD8$^+$ T cells and PD-L1, IDO
Expression of IDO and PD-L1 in B16 melanoma tumors growing in vivo depends on host CD8\(^+\) T cells and IFN-\(\gamma\)
Treg accumulation in B16 melanoma depends upon CD8$^+$ T cells but not IFN-$\gamma$.

- Treg recruitment appears to be regulated by chemokines (CCL22/CCR4).
- Also, no evidence for CD8s promoting migration or conversion.

Spaapen et al; manuscript submitted
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
• For IDO and PD-L1, IFN-\(\gamma\) is the major mediator in vivo
• For Tregs, CCL22 production by CD8+ effector cells is the major mediator, via CCR4 on Tregs (no evidence for Treg conversion or proliferation driven by CD8s)
• Blockade of these mechanisms represents attractive strategy to restore anti-tumor T cell function and promote tumor rejection in patients, and because these are intrinsic to the host they may be less mutable
• Clinical studies ongoing with anti-PD-1, IDO inhibitors, Treg targeting via CD25, and anergy reversal with homeostatic cytokines: already showing promise
Focusing in on T cell anergy

- A hyporesponsive state induced by TCR engagement in the absence of B7 costimulation
- Indirect evidence for involvement in tumor escape
- Functional overlap with “exhaustion”
- After anergy induction
  - T cells show defective TCR/CD28-induced Ras pathway activation (Fields et al. Science 1996) and blunted IL-2 production and proliferation
- Mechanism of anergy induction
  - Unbalanced activation of NFAT over AP-1 pathway: induction blocked by CsA, therefore is NFAT-dependent
  - Depends on new protein synthesis → induction of negative regulators
  - Recently identified diacylglycerol kinases (DGKs) as key inhibitors of Ras-mediated signaling in anergic cells (Zha et al Nature Immunol. 2006)
Further insight into T cell anergy: regulation by Egr2 driving DGK-α/ζ

Fields et al, Science 1996
Zheng et al, JEM In Press
Egr2 deletion leads to resistance to anergy induction \textit{in vitro and in vivo}

- CAR Tg x Egr2^{fl/fl} clones EV and Cre
- Treat cells Control plate-bound anti-CD3 (Anergic)
- Rest 1-2 days In medium
- Rechallenge with plate-bound anti-CD3+anti-CD28

**IL-2**

Control EV Control Cre Anergic EV Anergic Cre

**Zheng et al, JEM In Press**
T cell-intrinsic dysfunction (anergy): Strategy to determine global Egr2-driven transcriptional program in anergic T cells

- CAR Tg x Egr2fl/fl T cells: EV versus Cre
- Treat cells: Control versus plate-bound anti-CD3 (Anergic)
- Affymetrix gene expression profiling
- ChIPseq with anti-EGR2
- Merge datasets & Confirmatory qRT-PCR, ChIP assays

46 genes identified, including several surface proteins: LAG3 and CRTAM
Lag3 and Crtam are highly upregulated on a subset of CD8^+PD-1^+ TILs in B16 melanoma
Lag3^{+}\text{Crtam}^{+} \text{CD8}^{+} \text{TILs are defective in IL-2 production upon ex vivo stimulation}

The CRTAM+LAG3+CD8+ T cells also have blunted proliferation and express EGR2 and anergy-associated genes

*Zheng et al, manuscript submitted*
Tumor-infiltrating CD8⁺ T cells (brown) in human melanoma are EGR2⁺ (blue)

Implies that strategies to inhibit EGR2 pathway or target genes may have the potential to improve T cell function in human tumor context
Conclusions

• A T cell-inflamed tumor microenvironment may be a predictive biomarker for response to immunotherapies
  – Prospective analysis ongoing in GSK-Bio vaccine trials
• Innate immune “sensing” of tumors appears to occur via a STING-dependent pathway and host type I IFNs
• “Inflamed” tumors likely are not rejected due to dominant immune suppressive mechanisms
  – IDO, PD-L1, Tregs, Anergy: We can target these!
• Increased PD-L1, IDO, and Tregs in the tumor site are driven by CD8⁺ T cells in the tumor microenvironment
• A new set of surface markers driven by EGR2 may provide a strategy for identifying intrinsically dysfunctional CD8⁺ T cells from the tumor microenvironment, and may also regulate the anergic phenotype and be therapeutic targets
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Genetic melanoma model
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