The Immune Escape Game 2012: Targeting Tumor-derived Death Ligands, Exosomes and Treg

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Richard V. Smalley, MD Memorial Award Lecture 2012
Disclosure slide

• The presenter of the Richard V. Smalley, MD Memorial Award Lecture 2012 at the Annual SITC Meeting has no disclosures and no conflicts to report
Tumor

Pre-malignant Lesion

Advanced Oncogenesis

Tumor Growth

Elimination

Equilibrium

Escape

Immune System

Immune-surveillance

Immune selection

Immune subversion

Tumors are not passive targets for immune cells: they escape, prosper and fight back

- T-cells, especially TIL, are functionally impaired in tumor-bearing hosts
- Tumor-induced apoptosis of T/NK cells is seen in situ and in the circulation
- Tumors engineer immune escape and hide from the host immune system: appearance of “epitope-loss” variants which are resistant to immune cells
- Tumors create a unique microenvironment which promotes tumor growth and blocks anti-tumor activities of immune cells
Tumor microenvironment

Pro-inflammatory Activating signals

Death-inducing signals

Death-inducing signals

Tumor-derived inhibitory factors

ROS Inhibitory cytokines PGE₂

Defects in APM Immature phenotype

Cytokine imbalance Signaling defects

Tumor-derived inhibitory factors

Death-inducing signals

Growth-promoting signals

TAM

Pro-inflammatory Activating signals

ROS Inhibitory cytokines PGE₂

Tumor-derived inhibitory factors

Death-inducing signals

Growth-promoting signals

TAM

ROS Inhibitory cytokines PGE₂

Tumor-derived inhibitory factors

Death-inducing signals

Growth-promoting signals

TAM
Lymphocytic infiltrates in human tumors

CD3+, CD4+, CD8+, FOXP3+
Immune cells infiltrating a human tumor create its unique “immune signature” and mediate pro-tumor or anti-tumor functions depending on locally-generated signals.

Tumor elimination

Tumor infiltrating lymphocytes (TIL):
- CD8+CTL; CD4+Th2; CD4+Th17
- CD4+CD25+FOXP3+Treg
- CD3-CD56+CD16+ NK cells
- CD3+CD56+ NKT cells
- B cells
- Dendritic cells
- Myeloid-derived suppressor cells (MDSC)
- Macrophages (TAM)
- Granulocytes (PMN)
- Mast cells

Tumor progression

Prognosis??
Outcome?

Immune Score
Tumor cell

- TGF-β1
- IDO
- PGE₂
- VEGF
- GM-CSF
- MHC class II

DC

- B7-H1
- IL-6
- TGF-β1

Tumor progression

- Naïve CD8+
  - TCR
  - Perforin
  - Granzyme B
- CTL
- MHC class I

- MDSC
  - IDO
  - ROS
  - iNOS
- T2-type CD4 T cells
- Regulatory CD4 T cells
  - IL-4
  - IL-13
  - IL-10
  - TGF-β1
Mechanisms used by tumor cells to suppress anti-tumor immunity are many:

- Elimination of anti-tumor effector T cells in situ or in the periphery by using the death receptor-ligand signaling pathways
- Tumor-derived exosomes (TEX) as vehicles for the delivery of immunosuppressive signals
- Expansion of Treg in the tumor microenvironment and their impact on anti-tumor responses
What is happening to anti-tumor effector T cells during cancer progression?

Clues from studies of TIL isolated from human solid tumors:

- Low or absent $\zeta$ chain expression
- Suppressed NF$\kappa$B activation
- Depressed locomotion, proliferation, cytotoxicity
- Altered cytokine profile: a lack of IL-2 or IFN-$\gamma$
- Increased levels of caspase-3 activity
- Apoptosis of CD8+ T effector cells *in situ*
Apoptotic CD8+ T cells in the nest of lymphocytes at the tumor site

Red = alive CD8+ T cells  Blue = dying CD8+ T cells
FAS expression on and ANXV binding to CD8+ T cells in patients with HNC and NC

% CD3+ Fas+

Patients | N=35

% CD8+ Anx+

Patients | N=17

Controls | N=30

p < .0001

p = .0003
Apoptosis of circulating CD8+ T cells discriminates patients with HNC from normal controls.

P < 0.0001

However, this study also shows that Annexin binding does not discriminate between HNC patients with AD vs. NED.
Which subsets of circulating \textbf{CD3+ T cells} undergo apoptosis? (i.e., are ANXV+)

\textbf{CD8+} T cells preferentially over \textbf{CD4+} (>35\% vs. 5\%)

- \textbf{CD8+CD95+} (>50\% vs. <20\%)
- \textbf{CD8+CD45RO-CD27-} (>12\% vs. <2\%)
- \textbf{CD8+CD28-} (>15\% vs. <5\%)
- \textbf{CD8+CCR7+} (>35\% vs. <5\%)

The data are mean values from various cohorts of HNC patients vs. NC. All differences were statistically significant.

Is apoptosis \textbf{CD8+} T cells directed at clonally-restricted T cells mediating anti-tumor responses?
Restricted Vβ2 subset and ANX V binding to CD8+T cells in the circulation of a patient with HNC

ANXV binding to circulating CD8+ p53_{264} TET+ or CD8+TET- cells in patients with HNC

Expansion of CD8^{+}\text{GP100}_{209-217}^{+} T cells and change of differentiation status in this subset seen in one melanoma patient treated with a multi-epitope vaccine.

In HNC patients, the frequency of circulating CCR7+ CD8+ T cells is decreased, while the frequency of CCR7-CD8+ cells is increased.
Kaplan-Meier plots for DFS based on the frequency of CD8+CCR7+ T cells measured at the time of cancer diagnosis

- 25 patients (12 recurrences)
- 9 patients (1 recurrence)
- 16 patients (11 recurrences)

%CCR7+CD8+ ≥ 28%
%CCR7+CD8+ < 28%

p = 0.0115

Months After Definitive Therapy
Probability of Disease-Free Survival

High Risk
Low Risk
Fate of immune cells in patients with cancer

- Imbalance of lymphocyte subsets
- Selective demise of CD8+ effector T cells
- Accelerated apoptosis in tumor antigen-specific (tetramer+) T cells
- Persistent alterations in lymphocyte homeostasis: a rapid turnover
- Presence in serum of microvesicles “armed” with biologically-active FasL
The Fas/FasL pathway and T-cell apoptosis in situ or in the periphery

Tumor

FasL

sFasL

sFasR

FasR

MHC

TA

TCR

1.

2.

TIL or CD8+ T

Apoptosis

Whiteside TL.
Exosomes isolated from the serum of a patient with cancer, fixed with gluteraldehyde embedded in Epon and sectioned.

Transmission electron microscopy.
Sera of cancer patients and supernatants of tumor cells induce apoptosis of T cells

Exosomes isolated from sera of cancer patients induce apoptosis of T cells

Wieckowski E et al., J. Immunol 183:3720,2009
TMV carry immunosuppressive cytokines TGF-β1 and IL-10

Szajnik M, et al, PLoS ONE 5, 2010:
The diagram illustrates the interaction between Tumor cells and immune cells, specifically T cells, Treg cells, NK cells, Monocytes, and Immature DCs.

- **Tumor Cell**: Releases MV (Microvesicles).
- **Anti-tumor T cells**: Apoptosis of Fas+ TRAILR+ T cells.
- **FasL**, **TGF-β**, **TRAIL**, and **PGE2** are involved in the regulation of apoptosis and immune functions.
- **Monocytes**: Release PGE2 and may contribute to the suppression of T cell function.
- **Immature DCs**: Functions blocked by TGF-β.
- **NK Cells** and **Cytotoxic T Cells** are present, indicating a typical immune response.

The diagram highlights the complex interactions and the roles of various molecules in the immune response to tumor cells.
Exosome fractions obtained from sera of patients with Stage II or Stage III melanoma at the time of diagnosis and prior to therapy.
Exosome fractions obtained from sera of patients with melanoma prior and post therapy with high-dose interferon-α
Tumor-derived exosomes (TEX, TuMV) promote proliferation of human Treg


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Treg in humans

- Phenotype: $\text{CD4}^{+}\text{CD25}^{\text{hi}}\text{FOXP3}^{+}$
  $\text{CD4}^{+}\text{CD39}^{+}\text{CD25}^{+}\text{FOXP3}^{+}$

- Functional definition

- Different subtypes: nTreg
  inducible (i)Treg (Tr1)

- Treg accumulate in tumors, and their frequency is increased in the blood of cancer patients
Treg can be isolated from tissues and blood using surface markers such as CD25 or CD39

In OvCa, increased Treg frequency and function are associated with poor prognosis (e.g., Curiel et al, Nature Med:10, 2004 and many others)

In colon Ca, increased Treg frequency is associated with better prognosis and improved overall survival (Salama et al, J. Clin Oncol. 27, 2009)
Treg are recruited to the tumor site, they expand and suppress anti-tumor immune responses favoring tumor escape.
Mechanisms of suppression mediated by human regulatory T cells

Do all Treg use these mechanisms or are there functionally-specific Treg subsets?
Control (n=20)  
HNSCC (n=57)  

P = 0.01

a. CD25  
NC  
3%  
HNSCC  
9%

b. CD39  

% CD39+CD25+ Treg  
P < 0.005  
% CD39+CD25neg  
P = 0.01  

Control (n=20)  
HNSCC (n=57)  

R² = 0.8  
P < 0.005

c. % CD4+CD39+CD25+
**CD4+CD39+ subsets in human peripheral blood**

- Suppression via PD1
- Suppression via adenosine
- Suppression via TGF-β
- down-regulation of FOXP3??
- Conversion (IL-2; TGF-β)?
Treg in cancer clinical trials

- Treg (CD4+ CD25+FOXP3+) frequency is often serially monitored in immunotherapy clinical trials
- Attenuation of Treg could serve as measure of favorable response to therapy
- Unexpectedly, Treg were found to be significantly increased in the frequency and activity after various immunotherapies (data from many clinical trials and institutions)
Persistent elevation of Treg in the peripheral circulation of patients with HNC who are NED following chemotherapy or chemo-radiotherapy

AD = 15
NED = 25
Circumventing Treg activity in cancer??

- Elimination of Treg prior to immunotherapy
- Inhibition of Treg function
- Treg depletion:
  - Daclizumab (anti-CD25)
  - Ontak (denileukin difftitox)
  - Anti-CD25/toxins
  - TKIs (Sunitinib)
  - Chemotherapy drugs (?)
Activated Tr1 cell

- T effector cell 1

- Adenosine
  - A2AR

- COX-2

- CD4
- CD25
- CD39
- CD73

FUNCTION RESTORED

Disarming iTreg by blocking adenosine production

Disarming iTreg by blocking adenosine binding to A2AR

Disarming iTreg by blocking PGE2 production

Whiteside TL. CII 61: 283, 2012
Are Treg good or bad in cancer?

**GOOD**
- Treg
- G
- T
- B
- M

**BAD**
- Treg
- CTL
- Th
- NKT
- NK

**Inflammation**
(Tissue damage; Promotion of tumor growth)

**Anti-tumor immunity**
(Responsible for tumor demise)
Strategies for inhibiting the inhibitors

DC

MHC class I

MHC class II

B7-H1

IL-6

TGF-β1

1

2

3

4

5

6

7

CD8+

T effector

MHC class I

MHC class II

TGF-β1

IL-10

IDO

PGE2

EGF

VEGF

GM-CSF

Adenosine

IL-10

TGF-β1

Adenosine

Perforin

Gr A,B

FasL

IL-10

TGF-β1

GrA,B

FasL

MDSC

deplete

CTL

Proliferation

MDSC

deplete

Treg

deplete

T effector

Proliferation

DC

TU

EGFR

VEGFR

PD-L1

1

2

3

4

5

6

7

FasL

Fas

CTLA-4

CD8+

T

DC

MDSC

T effector

T effector

Proliferation

CTLA-4

CD8+

T

DC

MDSC

T effector

Proliferation

CTLA-4

CD8+

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T effector

Proliferation

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Improving therapeutic effects of immune therapies

- Delivery of immune therapies to patients with less compromised immune responses
- Understanding of molecular pathways responsible for tumor escape and of tumor immune signature
- Improved knowledge of mechanisms engaged by immunotherapeutic agents
- Rationally selected combinatorial strategies used in the setting of minimal residual disease
And so…the 2012 immune escape games are in full gear, with many promising strategies available for use and in use in the clinic……

“Why, is it asked, does the supply of new miracle drugs lag so far behind, while the biology continues to move from strength to strength…….? There is still the conspicuous asymmetry between molecular biology and, say, the therapy of lung cancer.”

-Lewis Thomas,
The Lives of a Cell, 1978
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Figure 1B.

Tumor cell

DC

CD8+

MHC class I

MHC class II

TLR

TLR

Co-stim

Co-stim

EGFR

VEGFR

PD-L1

Proliferation/
Differentiation

Adjuvants

Cytokine/
Chemokine
Receptors

TCR

MDSC

T-effector

Treg

TLR

Cytokine/
Chemokine
Receptors

Proliferation/
Differentiation

ADOPTIVE T-CELL TRANSFERS
Immune escape in early 1980s

- T cells obtained from human tumors (TIL) failed to clone vs T cells from the blood or from tissues of normal donors and were ineffective in eliminating tumor targets (e.g., Miescher S., et al, J. Immunol. 138:4004,1987)
- TIL proliferation as well as anti-tumor activity were inhibited by tumor-derived factors whose nature was an enigma at the time
- Concurrently, Dr. Rosenberg at NCI was successfully expanding TIL for IT of patients with melanoma
- Studies in mouse models of tumor growth (~1990s) confirmed the concept of tumor-mediated suppression under the name of “immunoediting” (Dunn et al, Nat Immunol. 3: 991,2002)