Mechanisms of immune “escape” & immune inhibition

ESMO Preceptorship, 19. November 2014

Daniel Speiser, Dept. of Oncology & Ludwig Center, CHUV, University of Lausanne - Switzerland

Disclosures: My lab has financial support from Boehringer-Ingelheim (Germany)
Therapy of cancer patients:

Clinical benefit depends on CD8 T cells and tumor resistance.

Increasing innate immune activation, supporting spont. CTL activity and immunotherapy (weak to strong vaccines, checkpoint targeting, ACT) - "1st dimension"

Decreasing immune escape and resistance of tumor tissues - "2nd dimension"
The three main stumbling blocks for anti-cancer T cells

A scientific “check-list” for T cell based immunotherapy of cancer patients

1. Non-self anti-viral
   - positive selection (thymus)
   - deletion (thymus / periphery)
   - naive
   - acute infection
   - memory (Ag cleared)

2. Self / neo-self anti-tumor
   - tumor
   - Ag persistence
   - 1st stumbling block: low T-cell numbers and low TCR affinity
   - 2nd stumbling block: inefficient priming / boosting
   - 3rd stumbling block: T-cell suppression in the tumor microenvironment

Baitsch, Speiser et al, Trends in Immunology, 2012; 33:364
Mechanisms of immune “escape” & immune inhibition

A major focus of tumor immunologists is on mechanisms in the tumor microenvironment.

Basic immunology, and other fields of immune research is more strongly focused on systemic immune activation and inhibition.
Extrinsic mechanisms of hyporesponsiveness of anti-cancer T cells

Apart from the three main stumbling blocks highlighted in this review, T cell extrinsic mechanisms play major roles in the hyporesponsiveness of anticancer T cells. Here, we list such mechanisms of tumor cells (I) and their microenvironment (II).

I. Mechanisms of tumor cells (reviewed in [81])
- Antigen loss: shedding of surface antigens, MHC downregulation, alterations of the antigen presenting machinery affecting peptide trimming, transport or MHC binding
- Production and release of immunosuppressive factors, e.g., IDO, prostaglandin (PG)E2, transforming growth factor (TGF)-β, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF)
- Overexpression of antiapoptotic molecules, e.g., BCL-2, FLIP
- Expression of Fas ligand or its release in microvesicles inducing T cell apoptosis
- Secretion of soluble Fas or DcR3 binding Fas ligand on T cells and preventing apoptosis [82]

II. Factors in the tumor microenvironment

Regulatory T cells (Tregs): (reviewed in [83])
- Tregs may inhibit anticancer T cells by
  - Induction of T cell apoptosis via cell–cell contact
  - Disruption of T cell metabolism due to transfer of cAMP via gap junctions
  - IL-2 consumption via CD25
  - Blocking of Th1 responses due to production of IL-10 and TGF-β

Tumoral DCs (reviewed in [84])
- Downregulation of MHC-II and the co-stimulatory molecules CD80 and CD86
- Induction of T cell apoptosis due to IDO expression
- Shifting T cell responses from Th1 to Th2 polarization due to IL-10 secretion

Tumor associated macrophages (M2) (reviewed in [85])
- Blocking of Th1 responses due to production of IL-10 and TGF-β
- Recruitment of naive T cells, Th2 cells and Tregs due to production of chemokine CC ligand (CCL)17, -18 and -22

Myeloid-derived suppressor cells (reviewed in [86,87])
- Shifting T cell responses from Th1 to Th2 polarization due to IL-10 production
- Arg-1 and inducible NO synthase (iNOS) activity: Arginine depletion and production of reactive oxygen species (ROS) and reactive nitrogen oxide species (RNOS)
  - Loss of antigen recognition due to TCR nitration
  - Nitrosylation of signaling proteins and prevention of their phosphorylation
  - Decreased IL-2 production due to unstable mRNA
  - Blocking of TCR signaling due to CD3-ζ chain downregulation
  - Enhanced T cell apoptosis
- Blocking T cell activation due to depletion of cysteine
- Blocking T cell migration due to loss of CD62L expression

Lymphoid-like reticular network
- Recruitment of lymphoid-tissue inducer (LTi) cells by tumors expressing CCL21 and consequent formation of a lymphoid-like stroma within tumors favoring a tolerogenic microenvironment [88]

Cancer-associated fibroblasts (reviewed in [89])
- Secretion of factors supporting tumorigenesis and metastases, e.g., epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF)
- Secretion of chemokines attracting tumor-promoting immune cells, e.g., chemokine CXC ligand (CXCL)14

Baitsch, Speiser et al, Trends in Immunology, 2012; 33:364
Mechanisms of immune attenuation in metastases

CTLA-4, PD-1 and further inhibitory receptors
   (Tim-3, Lag-3, 2B4, BTLA, TIGIT, VISTA, KLRG-1, CD160)
Regulatory T cells ("Treg"): CD4+CD25+FoxP3+ T cells
Myeloid derived suppressor cells (MDSC)
Th2 polarization of CD4+ T cells, ILCs and NKT cells (IL-4, -5, -13)
Indoleamine dioxygenase (IDO, tryptophan ↓), arginase I
Prostaglandin E$_2$, cyclooxygenase-2
VEGF, TGF-β, IL-10, IL-35
Fas, TRAIL
MHC ↓, antigen ↓ on tumor cells
Schematic representation of inhibitory receptor co-expression according to differentiation status and physical location

Mechanisms of CTLA-4 and PD-1/PD-L1 targeting

Early immune response: T cell activation

Effector Phase

Tumor cells

Lymph node

Blood vessel

Peripheral tissues

Tumor

APC

MHC

TCR

B7

CD28

CTLA-4

1st signal

2nd signal

INHIBITION

ACTIVATION

Anti-CTLA-4 Ab
- Ipilimumab
- Tremelimumab

Anti-PD-1 Ab
- Nivolumab
- Lambrolizumab
- Pidilizumab

Anti-PD-L1 Ab
- BMS-936550
- MPDL3280A
- MEDI4736

**Activation and differentiation status strongly impact on expression of inhibitory receptors (iRs)**

Inhibitory receptor+ T cells are often:

1. **activated** (except CD160, KLRG1, 2B4, BTLA)
2. **differentiated** (except BTLA)
3. **functional**. Reduced IFNγ/TNFα only in CTLA-4+ / CD160+ cells (as measured in absence of iR-ligands on APCs / targets)

- Analysis of T cell functions is important (albeit challenging)
- Phenotyping of iRs is not conclusive in absence of activation and differentiation markers

Legat, Fuertes Marraco et al, Frontiers Immunol 2013
Functional competence of “exhausted” CD8 T cells

Virus clearance in mice:

- Acute infection
  - LCMV Armstrong
  - Memory T cell population
  - Pathogen exposure

- Chronic infection
  - LCMV clone 13
  - Phenotypically exhausted T cell population

Cytotoxic capability in melanoma patients:

- PBMC
- TILN

Utzschneider et al, Nature Immunology 2013; 14:603

Mahnke / Devevre et al. OncoImmunology 2012; 1:467
Functionally adapted CD8 T cells

T cells in chronic infection and cancer (with “exhaustion” phenotype) can have comparable functional competence as effector T cells.

Acute phase

Pathogen cleared

Memory cells

Acute phase

Prolonged effector phase

Late phase

Pathogen / antigen clearance

Pathogen / antigen persistence

Short-lived effector population

Long-term effector population

Memory-like cells (with exhausted phenotype)

Subsets differing in Eomes expression, correlating with distinct functional properties

T-bet<sup>hi</sup> cells display low intrinsic turnover but proliferate in response to persisting antigen, giving rise to Eomes<sup>hi</sup> (PD-1<sup>hi</sup>) terminal progeny.

Genetic elimination of either subset results in failure to control chronic infection.
Driving malignant disease

Cancer tissue

TME

Cancer cell
The 2 backbones of malignancy:

1. **Cancer cell-internal** disease mechanisms

   Cancer cells “specialize” (by mutation & selection) through the acquisition of cancer cell-internal drivers:

   - Proliferative potential
   - Growth factors, & independence thereof
   - Survival & resistance to cell death
   - Adaptation to low oxygen & nutrition, metabolic & energetic adaptation

   Mutations +++