From basic to tumor immunology for oncologists

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DISCLOSURE information - Pedro Romero

Personal financial interests

• Speaker honoraria: BMS, Astra Zeneca, Roche
• Member Scientific Advisory Board: Immatics biotechnologies, Enterome
• Grant for research: Roche pRED, Zurich

Non-financial interests

• Transgene - Member Scientific Advisory Board
• NexImmune - Member Scientific Advisory Board
• Editor-in-chief - Journal for Immunotherapy of Cancer
«For their discovery of a revolutionary approach to cancer treatment» Nobel Committee, Stockholm, October 1 2018

James P. Allison  Tasukku Honjo
Concept of immunosurveillance
The current model of immunoediting: the three Es

**Elimination**
- *Cancer immunosurveillance*
  - Effective antigen processing/presentation
  - Effective activation and function of effector cells
    - e.g. T cell activation without co-inhibitory signals

**Equilibrium**
- *Cancer dormancy*
  - Genetic instability
  - Tumour heterogeneity
  - Immune selection

**Escape**
- *Cancer progression*
  - Tumours avoid elimination through the outgrowth of tumour cells that can suppress, disrupt, or ‘escape’ the immune system
Recognition of tumors by the immune system

What are the tumor targets recognized by T and B cells?
Structure of physiological antigen receptors

B CELL RECEPTOR (BCR)  T CELL RECEPTOR (TCR)

<table>
<thead>
<tr>
<th>T Cell Receptor</th>
<th>Antibody</th>
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<tbody>
<tr>
<td>Membrane</td>
<td>Membrane and soluble</td>
</tr>
<tr>
<td>Two chains</td>
<td>Two H and L chains</td>
</tr>
<tr>
<td>CDR1, 2 and 3</td>
<td>CDR1, 2 and 3</td>
</tr>
<tr>
<td>Ligand: MHC/peptide also unrestricted</td>
<td>unrestricted</td>
</tr>
<tr>
<td>Two isotypes: $\alpha\beta$, $\gamma\delta$</td>
<td>Five classes: M, G, A, D, E Several isotopyes, per class</td>
</tr>
<tr>
<td>Low affinity ($10^{-5}$ M) No affinity maturation</td>
<td>High affinity ($10^{-9}$ – $10^{-11}$) Affinity maturation</td>
</tr>
</tbody>
</table>
Engineering T cell antigen specificity by gene transfer

M Kalos & C June, Immunity 2013
CYTOLYTIC CD8 + T LYMPHOCYTES (CTL),
the major effectors of anti-tumor adaptive immunity
- Direct lysis (perforin, GZB)
- IFN-\(\gamma\), TNF-\(\alpha\), GM-CSF …
- Fas L
- CD40L
Molecular interactions involved in antigen recognition by cytolytic T lymphocytes (CTL)

The MHC Class I Processing and Presentation Pathway

- Plasma membrane
- Constitutive secretory vesicle
- Golgi complex

- Cytoplasm

- ER
- TAP
- β2m

- MHC class I

- Calnexin

- Cytoplasmic protein

- Proteasome

- Processed antigen
High Accuracy MS-based Identification of Human Leukocyte Antigen Class I Peptidomes

A: Sample preparation

Cells lysate → Immuno-affinity purification → Purification and enrichment → HLA-I complexes → HLA-I peptides

B: Liquid chromatography - mass spectrometry

HPLC → Quadrupole Orbitrap mass spectrometer → High resolution and high accuracy

C: Identification of HLA-I peptides

1. Unspecific database search using MaxQuant
   1% FDR threshold
   1,2,3 charge states
   Length of 8-15 a.a.

3. 45,000 MS/MS spectra

D: Define HLA-I consensus binding motifs

HLA-I peptides sequences

Define consensus binding motifs

Cluster of sequences using GibbsCluster tool

Bassani-Sternberg M

M & CP 2015
Thymic anatomy and compartmentalized selection, or the I-O preceptorship for T cells

Cortex

Medulla

Subcapsular epithelium

Capsule

Positive selection only

Positive and negative selection

Negative selection only

Compartmentalized selection

Non-compartmentalized selection

+ selection

Negative

(95%, RIP)

Palmer, Nat Rev Immunol 2003
Thymic selection depends on T-cell receptor affinity for self peptide–mHC complexes

Palmer, Nat Rev Immunol 2009
Aire: from transcriptional regulation to tolerance induction

Mathis & Benoist, Nat Rev Immunol 2007
CTL-defined tumor antigens

1- Shared, tumor specific antigens (e.g: MAGE-A3, NY-ESO-1)
2- Differentiation antigens (e.g: gp100, PSA, CEA)
3- Overexpressed antigens (e.g: HER-2/neu, WT-1)
4- Mutated, unique antigens (e.g: MUM-1, idiotypes)
5- Virus encoded antigens (e.g: HPV-16 E6/E7, HBV, HCV)

https://caped.icp.ucl.ac.be/
> 300 T cell defined tumor antigens, short peptides
A new generation of tumor specific antigens: the « neoantigens »

Somatic mutations

No central tolerance

Highly individual

NGS – Bioinformatic pipeline for identification

Schumacher & Schreiber, Science 2015
PURPOSE

• To compare the tumor neoantigen landscape between primary lesions and matched metastases in lung cancer

STUDY DESIGN

Variants selection:
tumor specific, AF≥0.05, DP≥10, mutation reads≥3
Neoantigen prediction:
high affinity to HLA (IC_{50}<500nM), fold change > 10, peptide length 9-10

Efficiency of prediction < 3%
Validation via immunopeptidomics, T cell recognition assays
The T-cell Anti-Tumour Response

1. Tumour antigens released by tumour cells

2. Tumour antigens presented to T cells

3. T cells are activated and proliferate

4. T cells recognise tumour antigens

5. T cells kill tumour cells

T cell priming

- A rare event
- Only one specialized APC may initiate a T cell response: XP-DC
- Highly localized event: lymph node (TuDLN)
- Takes a certain affinity of receptors
- Takes time
- At least three signals – highly regulated
Activation of Naïve T cells

- T cells require multiple signals (minimum of THREE) to become fully activated
- In addition to antigen stimulation further positive co-stimulation is required
- Key co-stimulatory receptor is CD28

\[\text{MHC} = \text{major histocompatibility complex}\]

\[\text{TCR}\]

\[\text{CD8}\]

\[\text{Co-stimulator}\]

Key Effector Cells Involved in an Anti-Tumour Immune Response

**CD8+ Effector T cells**
- Destroy tumour cells

**Th1 CD4+ Helper T cells**
- Produce cytokines that mediate inflammatory and effector responses;
- Help B cells make antibody; modulate CTLs

**Natural Killer cells**
- Destroy antibody-coated tumour cells or tumour cells lacking MHC I

**Key cytotoxic effector molecules**
- Perforin
- Granzymes
- Granulysin
- Fas ligand
- IFN-γ, TNF-α

**Key effector molecules**

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<thead>
<tr>
<th><strong>Th1</strong></th>
<th><strong>Th2</strong></th>
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<tr>
<td>CD40 ligand</td>
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**Macrophage presenting tumour antigen**

**B cell presenting specific antigen**

MHC = major histocompatibility complex; NK = natural killer; TCR = T cell receptor; IFN = interferon; GM-CSF = granulocyte macrophage-colony stimulating factor; TNF = tumour necrosis factor; IL = interleukin; CTL = cytotoxic T lymphocyte; Th = T helper cell

Immunological synapse: recognition is a two step process that delivers 2 signals to the T cell
Immune checkpoint CTLA-4

- Induced shortly after productive T cell activation

- High levels in regulatory T cells and in exhausted T cells

- Inhibits T cell functions, stops cell cycle progression

- Engaged by CD80 and CD86, the affinity of the interaction is 10 times higher than their affinity for CD28

- Critical for the fine tuning the priming of T cells - centrally
CTLA4 Negatively Modulates T-Cell Activation

CTLA4 Negatively Modulates T-Cell Activation

Dendritic cell

MHC

Antigen

TCR

CD28

B7

CTLA4

T cell
Blocking Antibodies to CTLA4 Allow Positive Signaling from Costimulatory Molecules to T Cells

Lymphocyte trafficking, extravasation and TILs
Hot tumors

Cold tumors
Therapeutic strategies to promote efficient T cell migration, extravasation and infiltration of tumors
Understanding the cellular and molecular bases of tumor infiltration by T cells

- Catenin signaling prevents migration of effector T cells into tumors

Intra-vital imaging revealed failed T cell entry into β-catenin expressing tumors

Effector T cell migration depends on the presence of CD103+ DCs producing CXCL10 (XP-DCs)

Lack of CD103+ DC-mediated effector T cell recruitment prevents immune control

Spranger S et al. Cancer Cell 2017
TGFβ in colorectal and urothelial carcinoma drives immune evasion by contributing to exclusion of T cells

Mariathasan S et al. Nature 2018

Tauriello DVF et al. Nature 2018
T cell exhaustion

- Progressive loss of T cell functions (lysis, cytokines, survival)

- Associated to chronic stimulation by antigen (signal 1)

- Typical phenotype: a constellation of co-inhibitory receptors (i.e. PD-1, TIM-3, CTLA-4, LAG-3, TIGIT, 2B4, VISTA ...)

- May develop side by side with anergy (lack of costimulation)

- May be compounded by immunosuppressive factors in the tumor microenvironment: TGFβ, IL-10, depletion of critical amino acids such as Trp (IDO), Arg (Arginases), Phe (IL4I1)
The PD-1 immune checkpoint

- Induced shortly after productive activation of naive T cells
- Overexpressed as a function of repeated antigen exposure
- A hallmark of both activated T cells and exhausted T cells. In both cases, ligation of PD-1 inhibits T cell functions
- Two ligands: PD-L1 and PD-L2
- PD-L1 is induced by IFNs in any cell type. It is also induced by oncogenes, and in Hodgking lymphoma by the chromosomal translocation
- PD-L2 is expressed only on dendritic cells and macrophages
- The PD-1/PD-L1 physiological role: limit immunopathology (protection of PERIPHERAL tissues from activated effector T cells)
PD-1/PD-L1: the backbone of modern cancer IT

LaFleur M et al. J Immunol 2018
Emerging immune checkpoints: immunometabolism

- A growing number of catabolic enzymes: IDO, arginases, phenylalanine oxidase (IL4I1), glutaminase (GLS)

- Targeting the purinergic pathways in the tumor microenvironment (CD39, CD73, adenosine receptors)

- Agenda for 2018 - 2095