Tumor Antigen, Tumor Immunogenicity and Immunization

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CYTOLYTIC T LYMPHOCYTES (CTL),

the major effectors of anti-tumor adaptive immunity

- Direct lysis (perforin, GZB)
- IFN-γ, TNF-α
- Fas L
Topics

Antigen recognition

Antigen processing

The CD8 T cell response

Eliciting CD8 T cell responses by vaccination
Antigen Recognition by Antigen-Specific CD8 T Cells

Antigen presenting cell

MHC class I

TCR

Peptide: 9 – 10 a.a.

CD8

CD8 T cell
Thymic selection depends on T-cell receptor affinity for self peptide–MHC complexes

Palmer & Naeher, Nat Rev Immunol 2009
In melanoma, antigen-specific CD8 T cells often develop, but their protective activity is limited by the relative lack of high affinity TCRs against self- (tumor) antigen.

Multiple approaches to enhance the avidity of antigen recognition by tumor reactive T cells

Designed by N. Rufer et al.
Approaches to identify T cell-defined tumor antigens

1- Genetic: molecular cloning guided by T cells

2- Biochemical: isolation and sequencing of antigenic peptides

3- Reverse immunology: web-based algorithms
T cell-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)

http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm

> 300 T cell defined tumor antigens, short peptides
Pathways for processing and presentation of MHC class I-restricted tumor antigens

- Aberrant transcription or mRNA splicing
- Translation of alternative or cryptic ORFs
- Post-translational modifications
- Splicing fragments that are originally non-contiguous in the parental protein
- Cytosolic proteases, other than the proteasome, produce or destroy antigenic peptides
Model of the peptide splicing reaction inside the proteasome

Vigneron & van den Eynde, Cell Mol Life Sci 2011
Determinants of peptide antigenicity

- TCR contacts
- Altered peptide ligands (APLs)
- MHC anchor residues
- MHC variant peptides (MVPs)

Edwards & Evavold, Immunol Res 2011
Melan-A/MART-1 MVPs

A27L  E L A G I G I L T V  \( \uparrow \)  \( \uparrow \uparrow \uparrow \)

A28L  A L G I G I L T V  \( \uparrow \)  \( \downarrow \downarrow \downarrow \)
Natural decapeptide

E A A G I G I L T V

Decapeptide analog

E L A G I G I L T V

Sliz P et al. JI 2001
Tumor antigenic peptide MVPs in clinical or preclinical testing

- gp 100 (melanocyte/melanoma)
  - IMDQVPFSV
  - YLEPGPVT

- CEA (epithelial carcinomas)
  - YLSGANL

- NY-ESO-1/LAGE-1 (cancer/testis)
  - SLLMWITQA
  - SLLMWITQ
Monitoring the tumor antigen specific CD8 T cell response
Six major CD8\(^+\) T-cell subsets.... in humans
Differential lytic effector function displayed by EM28+ and EM28- T cell subsets

gene expression by 5 cell RT-PCR

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<tr>
<th></th>
<th>N</th>
<th>EM28+</th>
<th>EM28-</th>
<th>E</th>
<th>-</th>
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<tbody>
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<td>Granz B</td>
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<td>Perforin</td>
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<td>IFNγ</td>
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protein expression by intracellular staining

N

EM28+

EM28-

Eff

Granzyme B

Perforin

Romero et al. JI 2007
Monitoring a CTL response to a well defined tumor antigen

Enumeration and phenotype by flow cytometry: Tetramers$^{\text{PE}}$, CD45RA$^{\text{APC}}$, CCR7$^{\text{FITC}}$

IFN-γ production by flow cytometry (intracellular, secretion)

ELISPOT

Lytic activity

TCR usage

Functional avidity

Replicative history: telomere length (Flow-FISH); content of TCR rearrangement excision circles (TRECs)
The melanocyte/melanoma differentiation antigen: Melan-A/MART-1

- 118 a.a., apparent MW 22-24 kD
- Melanocyte lineage specific
- Function unknown
The Melan-A/MART-1 specific CD8 T cell repertoire in healthy HLA-A2 individuals

- One in 1,400 blood CD8 T lymphocytes are specifically labeled with fluorescent multimers

- Phenotypically and functionally they are naive T cells

- High level of positive selection in human thymus

- They undergo limited expansion in the peripheral immune system

- Unique example of « immunological ignorance » in humans

Pittet et al. 1999; Zippelius et al. 2004
Ex vivo detectable HLA-A2/Melan-A specific CD8 T cells in humans

Mean = 0.08 %
(n = 76)

Voelter V. ... Appay V, 2008
Metastatic (stages III and IV) melanoma patients (HLA-A2)
LAU 465 – Lymph node metastasis

H&E

Melan-A/MART-1

CD3

CD8
A2/Melan-A tetramer$^+$ cells in TILNs ex vivo

~ 15 %

A2/gp100, A2/tyrosinase, A2/MAGE-10, A2/LAGE and A2/NY-ESO-1 tetramer$^+$ cells were non detectable (<0.0 4%)
Evidence of Melan-A specific response in two thirds of metastatic melanoma patients, accumulating in tumor lesions

Naïve

RA⁺CCR7⁺
CD27⁺CD28⁺

Memory

RA⁻CCR7⁻
CD27⁺CD28⁺⁻

Tumor sites

Hyporesponsive CD8 T cells at the tumor sites:
- Low IFN-γ release
- Low perforin content
- Reduced tumor cell lysis

- Reversible upon culture in presence of IL-2
Tumor antigen specific IFN-γ release is specifically blunted in tumor infiltrating lymphocytes.

Zippelius et al. 2004; Baitsch, Speiser et al. 2011
Exhausted gene set enrichment in Melan-A-specific CD8 T cells from metastatic lymph nodes

Baitsch, Speiser et al, 2011
Can we rekindle tumor antigen specific T cells by vaccination?
Subunit vaccines: defined molecular composition

Minimal number of molecularly defined vaccine modules

Target antigen (signal 1)

Immunological adjuvant (at least signal 2?, >)

Delivery vehicle
Cancer vaccines, the many ways to deliver Ags

Synthetic peptide: short, exact epitopes; or long peptides, multiple epitopes

Recombinant protein

Recombinant viral like particles (VLPs)

Naked, stabilized nucleic acids:
  DNA plasmids
  mRNA

Recombinant viruses (many candidates):
  Pox
  Adeno
  Retro, lenti
  Sindbis

Recombinant bacteria:
  Salmonella
  Listeria

Dendritic cells
TLR agonists

Activated T helper cell

Pathogenic microbes!

CD40 ligand

Nature’s adjuvant: mature dendritic cells

The late R. M. Steinman

J. Hoffmann & B. Beutler

NK cells

TLR agonists

Endogenous innate R. ligands (DAMPs)

Cytokines

Pathogenic microbes!
TLR agonists as vaccine adjuvants

**TLR-4**: LPS, MPLA present in various formulations (e.g. GSKBio adjuvants)

**TLR-3**: proven in preclinical models, ongoing in clinical trials

**TLR-7**: Aldara™ (Imiquimod) being tested in cancer patients

**TLR-9**: synthetic oligos bearing CpG motifs; the most potent in terms of adjuvanticity
### Results of sequential phase I clinical trials in patients with stage III/IV melanoma in Lausanne

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<th>Adjuvant</th>
<th>TLR agonist</th>
<th>Immune response</th>
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<tr>
<td>None</td>
<td></td>
<td>0/6</td>
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<tr>
<td>AS02B (GSK Bio)</td>
<td>TLR4</td>
<td>1/12</td>
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<tr>
<td>K. pneum. outer membrane P40 (P. Fabre) TLR2</td>
<td></td>
<td>0/9</td>
</tr>
<tr>
<td>Montanide (IFA, Seppic)</td>
<td></td>
<td>12/17</td>
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<tr>
<td>rtLAG-3 (Immutep)</td>
<td>DC activator</td>
<td>?</td>
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Molecularly defined therapeutic vaccines: synergy between IFA and TLR9 agonist

100 µg peptide (analog)

500 µg CpG-ODN (type B) [PF 676]

Montanide ISA 51, 1 ml

Monthly s.c. injections
Strongly increased T cell frequency after 4 vaccinations with Melan-A peptide + CpG 7909 + IFA (PF-3512676).

Ex vivo flow cytometry analysis of PBMC:

Before: <0.01%
2 vacc: 1.09%
4 vacc: 2.08%

Speiser et al. JCI 2005 115:739
Vaccination with CpG 7909 (PF 676) + peptide analogue + IFA:

- rapid and efficient T cell responses, frequency 10-fold higher than without CpG

- peak response 7 to 11 days after booster injection

- generation of (central and) effector memory T cells expressing perforin, granzyme B and IFN$_\gamma$

- progressive generation of CD28- effector T cells

- killing of melanoma cells, IFN$_\gamma$ secretion
Other peptide based vaccines extensively tested in melanoma:

MAGE-A3\textsubscript{168 – 176}, HLA-A1

NY-ESO-1\textsubscript{157 – 165}, HLA-A2

gp100\textsubscript{209 – 217} analog +/- high dose IL-2

(extended survival reported by Schwartzentruber 2009)
Sipuleucel-T (Provenge): autologous “APCs” cultured with antigen-cytokine fusion protein

Recombinant Prostatic Acid Phosphatase (PAP) fusion antigen combines with resting antigen presenting cell (APC)

APC takes up the antigen

Antigen is processed and presented on surface of the APC

Fully activated, the APC is now sipuleucel-T

INFUSE PATIENT

Infused sipuleucel-T activates T-cells in the body

T-cells proliferate and attack cancer cells

Ag: PAP

Cytok: GM-CSF
IMPACT Overall survival
Final analysis (n = 349 events)

36.5 mo median f/u
HR = 0.759 (95% CI: 0.606, 0.951)

p = 0.017 (Cox model)
Median Survival Benefit = 4.1 months

Hormone refractory metastatic prostate carcinoma
Other therapeutic cancer vaccines in use

Oncophage, Russia from 2008

Provenge, USA from 2010

Prostvac, USA on fast track
Two vaccines in randomized phase III clinical trials in patients with lung cancer

Rt MAGE-A3 protein (GSK Bio)

MUC-1 peptide (Merck – Biomira)
HPV16 E7 + E6 long synthetic peptide cocktail vaccine

Approx. 15 peptides (~25 – 20 amino acids)

N = 20 patients with VIN

10+ complete tumor regression (> 50%)

Correlation with overall specific immune response
Cancer vaccine development:

Frequent induction of vaccine specific T cell responses (40-80 %)

Sporadic tumor regression (3 – 10 %)

Favourable clinical outcome in even higher proportions of patients (up to 25 %)

Correlation with immune response
Two major weaknesses in current therapeutic cancer vaccines

**Low potency** – need for optimization

**Short lived responses** – memory formation, negative modulation at the tumor sites
PD-1 expression of trp2-specific CD8+ T cells after therapeutic rLV vaccination

Sierro et al. EJI 2011
Blockade of PD-L1/PD-L1 pathway and rLV-trp2: Combination therapy ameliorates tumor control
Cancer vaccine development:

Comprehensive immunomonitoring

Clinical endpoints, modified response criteria

Predictive biomarkers, in development:

e.g. ICAM-1 levels in infused cells – Provenge
Gene signatures, immune response genes

Vaccines combined with immunomodulators or standard tts
Anti-CTLA-4 mAb approved for melanoma in 2011
Thanks!