Dendritic Cell-based Immunotherapy Against Tumours

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Main Outlines

- Tumor immunotherapy – basis & rationales
  - Immunity against tumors – evidence
  - Immune escape mechanisms of tumors – obstacles
  - Tumor immunotherapy – types & approaches
- DC-based tumor vaccine
  - Aim & objectives
  - DC immunobiological properties revisited
  - Key concepts & hypothesis
  - Early studies in animal models
  - Further experimental studies & clinical trials
  - Problems encountered
  - Recent development & future direction

Immunity against tumors

Evidence: (clinical)

- Postmortem: Tumors > clinically diagnosed
- Neonatal or old age > Adults
- Spontaneous regression of tumors
- Graft Versus Leukemia (GVL) responses

Immunity against tumors

Evidence: (oncologic)

- Tumorigenesis
  - Genetic
  - Chemical carcinogens
  - Irradiation
  - Virus-induced (e.g. HBV, EBV, HPV…)
  - Immunodeficiency
- Mutations
  - Tumor-Specific Antigens (TSA)
- Aberrant expression
  - Tumor Associated Antigens (TAA)

Immunity against tumors

Evidence: (immunological)

- Immune cells infiltrate in & around tumors
- Types of immunity involved:
  - Innate: NK, MQ…
  - Adaptive:
    - Antibodies (B cells, humoral)
    - Cytotoxic & helper T cells (cellular)
- TSA & TAA
- Tumor preventive vaccination against viruses:
  - HBV – Hepatocellular carcinoma (HCC)
  - HPV – Cervical cancer

Immune escape mechanisms of tumors

- Tumor cells "sneak through"
  - TSA/TAA - Poor immunogens
  - Tumor growth (speed, size)
- Lack of molecules important in immunity
  - MHC Class I, II
  - Co-stimulatory molecules, cytokines…
- Tumor-derived factors suppress immunity
  - Immunosuppressive molecules, cytokines
  - TSA/TAA-specific T (and B) cells – anergized

Immunotherapies against tumors

- **"Passive" approaches:**
  - Immunogenic enhancers, cytokines:
    - e.g. IL-2, IFN-α
  - Monoclonal antibodies:
    - e.g. Anti-CD20, Herceptin (Trastuzumab)
  - Adoptive transfer of tumor-reactive lymphocytes:
    - e.g. ex vivo & in vitro expanded CTLs
- **Active immunization** (cancer vaccines):
  - Conventional immunization protocols
    - against oncogenic pathogens (e.g. viruses)
    - against identified TSA, TAA
  - **Dendritic cell-based tumor vaccines**

DC-based Tumor Vaccine

Aim: to elicit specific immunity of the host to destroy tumors

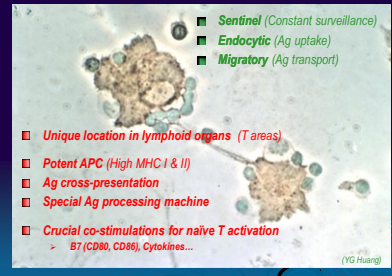
Approaches:
DC pre-loaded with tumor antigens
DC modified to express TSA/TAA…

Types of DC:
DC directly isolated from tissues
DC generated from precursors (PBMC, BMC…) in vitro

Types of tumor antigens:
Tumor lysate
Peptides (Purified or synthetic tumor peptides, or oncologic viral peptides)
DNA/RNA encoding TSA/TAA
DC-tumor cell fusion

Dendritic cells

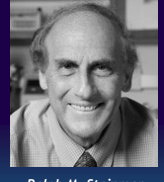
- The uniquely combined immunobiological properties



→ The true professional APC

Discovery & Pioneer Work on Dendritic Cells Honoured

2011 Nobel Prize in Physiology or Medicine
(Oct. 3, 2011)



Ralph M. Steinman
(Jan 14, 1943 — Sept 30, 2011)
Henry G. Kunkel Professor
The Rockefeller University

J Exp Med. 1973 May 1;137(5):1142-62.
J Exp Med. 1974 Feb 1;139(2):380-97.
J Exp Med. 1974 Jun 1;139(6):1431-45.
J Exp Med. 1975 Apr 1;141(4):804-20.
J Exp Med. 1979 Jan 1;149(1):1-16.

DC-based Tumor Vaccine

- **Original concept & hypothesis:** Lack of tumor Ag presentation is a major problem that can be bypassed by autologous DC preloaded with TSA/TAA.
  - ✓ T cells are most important in anti-tumor immune responses
  - ✓ DC are professional APC for T cell activation
    - T cell activation requires Ag presentation
    - T cell recognition of Ag is MHC-restricted
    - DC express high levels of both MHC Class I & II
    - DC may transport Ags to the sites of T cell activation
  - ✓ DC may effectively take up & process tumor Ags for presentation to T cells
- **Initial key objective:** to improve the efficiency of tumor Ag presentation.

Early studies in animal models

Influence of dendritic cells on tumour growth
(Knight SC, & Medawar PB et al., PNAS. 1985; 82:4495-7)

- Tumor model:
  - **Drug-induced Mouse sarcoma** (McSa-1)
  - DC isolated from spleen (spDC) & exposed to tumor Ags (lysate);
  - Injection (i.v.) of the tumor Ag-loaded DC into syngenic tumor-bearing mice;
- Some protection observed against tumor growth, but depending on the:
  - **dose of tumor Ag** DC exposed to (**low** > high);
  - **time of immunization** given (**early** > late)

Early studies in animal models

Immunity to a syngeneic sarcoma induced in rats by dendritic lymph cells exposed to the tumour either *in vivo* or *in vitro*

(Gyure LA, & Hall JG et al., ***Br J Cancer.*** **1987**; **55**:17-20)

- Tumor model:
  - ***Drug-induced Rat sarcoma*** *(HSN)*
- DC isolated from lymph (*L-DC*), HSN-sensitized *in vivo*
- Lymphocytes isolated from lymph, HSN-sensitized *in vivo*
- DC isolated from lymph, HSN-sensitized *in vitro*
- *Protection against tumor growth in syngenic naïve rats*

Early studies in animal models

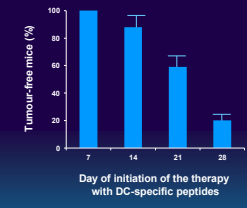
Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective & therapeutic anti-tumour immunity

(Mayordomo JI, & Lozte MT et al, ***Nat Med,*** **1995**. **1**:1297-302)

- Mouse tumor models
  - **Lewis lung carcinoma** (*3LL*)
  - **C3 sarcoma** (*HPV-16-transformed*)
  - **Melanoma** (*MO5 OVA-transfected*)
- Identified tumor epitopes (Class I-restricted):
  - **MUT1** (*LL carcinoma, H-2K^b restricted*)
  - **E7**$_{49-57}$ (*C3 sarcoma, H-2D^b restricted*)
  - **OVA**$_{257-261}$ (*Melanoma, H-2K^b restricted*)
- DC generation from bone marrow precursors (*BMDC*):
  - *in the presence of:*
  - **GM-CSF**
  - **GM-CSF + TNFa**
  - ***GM-CSF + IL-4***
- *In vivo* anti-tumor immunity
  - ***CTL-mediated***
  - ***Protective & therapeutic***

DC-based tumor vaccines

- Therapeutic immunity against established tumor in a mouse model



HPV-16-sarcoma
Tumor peptide: E7$_{(49-57)}$
BMDC (GM-CSF + IL-4)

(Mayordomo JI, & Lozte MT et al, *Nat Med*, 1995)

Essential DC-derived co-stimulatory signals

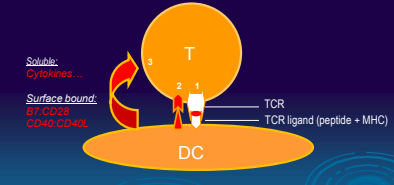
Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation, & Th1-associated cytokines

(Zivogel L, & Stortus WJ et al, ***J Exp Med.*** **1996**;**183**:87-97)

Dendritic cells as adjuvants for immune-mediated resistance to tumours

(Schuler G & Steinman RM, ***J Exp Med.*** **1997**;**186**:1183-7)

Crucial co-stimulatory molecules for T cell activation



DC-based Tumor Vaccine

- *Concept modified:*

  DC are potent APC for T cell activation:
  - may take up tumor Ags for presentation - *Signal 1*
  - *can provide crucial co-stimulatory signals – "Signals 2, 3…"*

  → ***An 'immunogenic' cell vector for vaccine delivery***

- *DC phenotypic & functional heterogeneity:*
  - DC lineages & subsets
  - DC maturity & functions

  → ***'immunogenic' vs 'tolerogenic' DC***

DC maturity & immuno-adjuvanticity

Immature DC
(Ag uptake mode)

Low surface MHC
Low B7

Mature DC
(Ag presenting mode)

High surface MHC
High B7, cytokines

'Tolerogenic'

'Immunogenic'

Molecular & Functional conditioning of dendritic cells for DC-based tumor vaccines

- ***DC functionally conditioned to enhance their immunogenicity:***
  - DC maturation & activation factors (e.g. LPS, HSPs…)
  - Immunogenic cytokines (e.g. IL-4, IL-12, IL-15, IL-18, IFN, TNF…)
- ***DC genetically modified to deliver full T cell activation signals:***
  - TSA/TAA-encoding DNA/mRNA
  - DC : Tumour cell fusion
  - B7 (CD80, CD86), CD40, CD40L, TLR ligands etc
  - Cytokines: IL-12, IL-15, IL-18, IFN etc
  - Chemokines

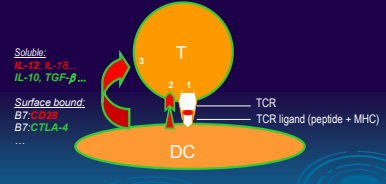
(Shurin MR et al, *Expert Opin Biol Ther.* 2010;**10**:1539-53)

Clinical Applications

- **Over 300 clinical trials conducted worldwide** (*last 2 decades*);
- **Tumours of different types & stages tested;**
- **Certain immune responses evident;**
- **Disappointing clinical outcome:**
  - *Low efficacy & consistency*
  - *Limited rate of objective tumour regression observed*
- **Problems encountered & possible explanations:**
  - *Suboptimal DC preparations*
  - *Lack of uniform production standards*
  - *Variations in protocol design & response monitoring*
  - *Quality of immune responses induced*
  - ***Cancer-related immunosuppression***

(Shurin MR et al, *Expert Opin Biol Ther.* 2010;**10**:1539-53)
(Palucka K et al, *J Immunol.* 2011;**186**:1325-31)

Crucial co-stimulatory molecules for DC-mediated T cell activation & inactivation



Interleukin-10

- *"Cytokine synthesis inhibitory factor (CSIF)"*
- A potent immunosuppressive cytokine
- Inhibit Th1 immunity (IFN-γ, IL-12), also down-regulate DC functions
- Produced by DC, macrophages, activated T cells, regulatory T & B cells, & even by certain types of tumor cells

(Moore KW et al. Science. 1990)

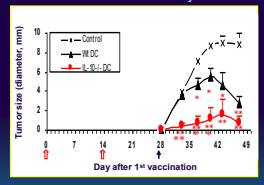
A crucial role for DC IL-10 in inhibiting successful DC-based immunotherapy: Superior Anti-Tumor Immunity against Hepatocellular Carcinoma Evoked by Dendritic Cells Devoid of IL-10

(Chen YX et al, *J. Immunol.* **2007**, **179**:6009-15)

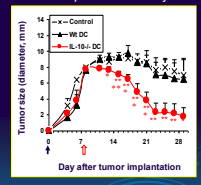
- Tumor model: ***Mouse liver cancer*** (Hepa 1-6)
- BMDC generated from IL-10KO mice are immunologically heightened:
  - *Express high surface MHC I & II*
  - *Produce more Th1 cytokines (IL-12, IFN-γ)*
  - *Stimulate strong T cell responses*
  - ***More resistant to the tumor-mediated immune suppression***
- *Superior anti-tumor immunity: Protective & therapeutic*

A crucial role for DC IL-10 in inhibiting successful DC-based tumor immunotherapy

Protective immunity



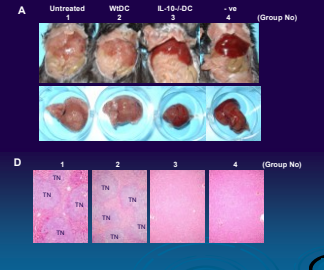
Therapeutic immunity



Extra-hepatic HCC model

(Chen YX et al, J. Immunol. 2007)

Superior anti-tumor immunity against mouse liver cancer evoked by DC devoid of IL-10



PV-HCC model (Wk-3)

(Chen YX et al, J. Immunol. 2007)

Human suppressor of cytokine signalling 1 (SOCS1) controls immunostimulatory activity of monocyte-derived dendritic cells

(Hong B et al, *Cancer Res*, **2009**, **69**:8076-84)

- SOCS1: a down stream regulator of IL-10R signalling
- A feedback inhibitor of JAK/STAT pathway
- SOCS1-silenced DC showed high immunogenicity:
  - *enhanced IL-12 expression*
  - *driving strong CTL-mediated anti-tumour responses*

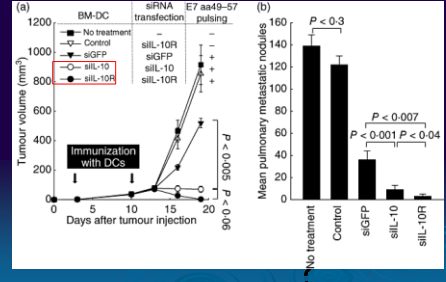
Blocking the immunosuppressive axis with small interfering RNA targeting interleukin (IL)-10 receptor enhances dendritic cell-based vaccine potency

(Kim JH et al, *Clin Exp Immunol*, **2011**;**165**:180-9)

- Tumour model: **E7-transform** mouse lung epithelial cells
- Direct blocking of IL-10R (by siRNA) enhanced DC immunogenicity:
  - *enhanced MHC class II, CD40 & IL-12 expression*
  - *resistant to rIL-10-mediated suppression*
  - *driving strong HPV E7-specific CD8+ T cell responses*
- *Superior therapeutic immunity against the E7 expressing tumour (TC-1)*

Vaccines delivered by DC knock-down of IL-10 or IL-10R induced strong anti-tumour immunity

Primary tumour *Lung metastasis*



(**Tumour model**: E7-transform mouse lung epithelia, s.c.)

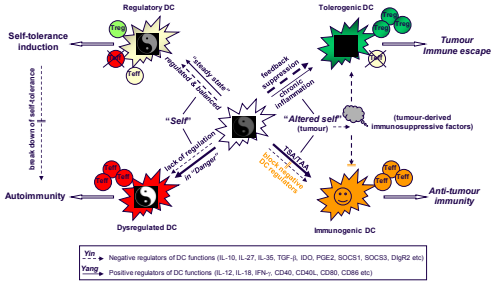
(Kim JH et al, *Clin Exp Immunol*, 2011;**165**:180-9)

Gene silencing of TGF-β1 enhances antitumor immunity induced with a dendritic cell vaccine by reducing tumour-associated regulatory T cells

Conroy H et al, *Cancer Immunol Immunother*, ***2011*** *Dec 23.* *[Epub ahead of print]*

- **Mouse colon carcinoma** (CT26) **tumour cells secrete TGF-β1**
- **Block CT26 TGF-β1 expression by siRNA:**
  - *inhibited TGF-β1 production in vitro*
  - *inhibited CT26 tumour growth in vitro & in vivo*
- **Co-injection of mice with TGF-β1 silencing-siRNA & tumour Ag-loaded DC:**
  - *Suppressed Treg*
  - *Enhanced effector T cell infiltration in the tumours*
  - *Induced both protective & therapeutic anti-tumour immunity*

DC-based Tumour Vaccines - *Targeting the Negative Arm of Immune Regulation*



Guiding the "misguided": Understanding the "*Yin*" and "*Yang*" of DC immunobiology. New insights from studies of the mechanisms underlying autoimmune responses indicate that the most effective way to enhance the DC immunogenecity is by alleviating the negative regulators of DC functions (e.g. DC devoid or knock-down of IL-10). This diagram illustrates the critical roles of DC in the induction and regulation of immune responses against "self", and "altered self". Treg: regulatory T cell; Teff: effector T cell; TAA: Tumour-associated antigens. (***Eur. J. Immunol. 2011**. 41:18-25*)

Summary I
DC immunobiological properties - revisited

Basic properties:

- ***Sentinel position/distribution*** *(Constant surveillance)*
- ***Endocytic activities*** *(Ag uptake)*
- ***Migratory nature****(Ag transport)*

Other unique features:

- *Unique location in the secondary lymphoid organs (T area)*
- High surface MHC Class I, Class II *(Ag presentation to Tc & Th)*
- *Co-stimulatory signals for T cell activation & inactivation (B7, cytokines…)*
- *Unique Ag processing capacity (Ag processing)*
- Ag cross presentation *(Th, Tc)*

→ ***A true professional APC***
→ ***A link between the innate & the adaptive immune systems***
→ ***A master regulator of the adaptive immunity***

Summary II
DC-based Tumour Vaccine

Aim: *To elicit specific immunity of the host to destroy tumors*

Basic concept & rationales:

- Evidence of immunity against tumours (*clinical, oncological & immunological*)
- T cells crucial in mediating anti-tumour immunity
- DC – Potent APC for T cell activation
- DC may take up, process & transport tumor antigens for presentation (*Signal 1*)
- DC can provide essential co-stimulatory signals (*Signals 2, 3...*) for T cell activation
- ***An 'immunogenic' cell vector for vaccine delivery***

Limitations:

- DC heterogeneity, functional plasticity & tolerogenic potential
- *Live cell approach - Susceptibility to the immunosuppressive tumour microenvironment*

Future prospects:

- *To improve the vaccine efficacy by targeting the negative regulators of DC functions*
- *To translate timely the new experimental findings for clinical applications*
- *To standardize the protocols for DC generation, vaccine design & response assessment*