Dendritic cells: from their Discovery to their Therapeutic Utility

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Outline

• Discovery of dendritic cells and their functional role in T cell priming (Ralph Steinman awarded Nobel in 2011)

• Role of Toll like receptors in regulating dendritic cells priming of T cells (Bruce Beutler and Jules Hoffmann, shared Nobel in 2011)

• Utilizing dendritic cells therapeutically
Dendritic Cell History

• In 1973, Ralph Steinman coined the term dendritic cell
• He described dendritic processes that form and retract, CDllc, and high MHC II, located throughout the body
What is the function of Dendritic Cells?

• Express high amounts of MHC I and II

Raised the question:
Can DCs stimulate a T-cell response?
Dendritic cells are very potent T cells stimulators

**FIG. 2.** Potency of purified DCs (Δ) vs. unfractionated spleen (○) in inducing an MLR. Responses (cpm of [3H]thymidine uptake – background/culture) were measured at various doses of stimulator cells, after 4 days of culture. Background was 5410 cpm.

Steinman, Witmer
PNAS (1978)
75:5132
Another Dendritic Cell Type

• Plasmacytoid DCs (pDCs)
  – Lack markers of other lineages, resemble plasma cells rather than have dendritic shape
  – Poor antigen presenters unless stimulated
  – Express BDCA2 (CLEC4) and Siglec-H
  – Have high levels of TLR 7,8,9
  – Large producers of IFNa

Dendritic Cell Development
DCs are the prime initiators - stimulate naïve T cells

- DCs present antigen to T cells and tell them whether to respond

- T effector responses such as cytotoxic responses, CTL, against viruses

- T helper responses to enhance antibody responses

What regulates when a DC stimulates a T cell?

- DCs communicate information to T cell via three major signals

- Signal 1 – antigen presented in MHC – “What should I respond to?”

- Signal 2 – costimulation – “Is is dangerous?”

- Signal 3 – Cytokine milieu – “What type of response should I make?”
DCs mature when they sense danger

• DCs provide Signals 1,2,3 best when they undergo “maturation” as a result of sensing the presence of microbes or tissue damage
  – So called danger signal

• Microbial motifs are sensed by Pattern Recognition Receptors (PRRs)
  – Toll like receptors (Bruce Beutler, Jules Hoffmann)
  – Nod like receptors
  – Other cytosolic receptos

Lemaitre Cell 1996
Poltorak Science 1998
Immature DCs induce T-cell tolerance

Banchereau J et al
Nature Reviews Immunology 5, 296-306
Mature DCs induce T-cell activation

Banchereau J et al
Nature Reviews Immunology 5, 296-306
Many TLR signals of Danger

• Conserved microbial motifs
  – Double stranded RNA
  – Lipopolysaccharide (LPS)
  – Unmethylated CpG DNA
  – Flagellin

• Products of tissue damage
  – HMGB1
  – S100B
  – Uric acid
DCs acquire antigen in many ways

Figure courtesy of Julie Blander
Signal 1

- Antigens are taken up by phagocytosis, cleaved into peptides, presented on MHC II to CD4+ T-cells
- Proteins produced in the cytosol, cleaved into peptides, presented on MHC I to CD8+ T-cells
- Signal 1 is proportional to:
  - The amount of available antigen (increased with increased pathogens)
  - The amount of MHC sent to the plasma membrane (increased upon maturation)
Optimization of Signal one with TLR Maturation

- Prior to maturation, most of the MHC II is located intracellularly in the multivesicular bodies
- Prior to maturation, DCs are good at phagocytosis, sampling their environment
- Upon maturation through TLRs, DCs stop phagocytosing, and send most of their MHC II to the plasma membrane at the time danger is sensed
Maturation of DCs

Signal 2

- Costimulators are upregulated upon maturation

- CD80 and CD86 – bind to CD28 on T cell and provide extra signals to enhance TCR signals, promotes Th1 response

- OX40L – binds to OX40 on T cells, promotes Th2 skewing
Signal 3

- Cytokines aimed toward “skewing” the T cell response
Type of Danger determines nature of Signal 3
DC GENERATE ANTI-TUMOR IMMUNITY

Acquisition of tumor antigens by DC

TUMOR BED

Tumor cells

Immature DC

Tissue factors released by dying cells (e.g. HSP, HMGB1)
DC GENERATE ANTI-TUMOR IMMUNITY

**Acquisition of tumor antigens by DC**

**TUMOR BED**

Immature DC

Tissue factors released by dying cells (e.g. HSP, HMGB1)

**Maturation of DC**

Mature DC

Upregulate HLA, costimulatory molecules and cytokines

**Migration to draining lymph nodes**

LYMPH NODE

CD4\(^+\) T cell

CD8\(^+\) T cell

CD8\(^+\) T cell

CD4\(^+\) T cell
Tumor peptide + HLA Class I/II

CD8$^+$ T cell

CD4$^+$ T cell

Differentiation of effector T cells

LYMPH NODE

DC GENERATE ANTI-TUMOR IMMUNITY

CD80/CD86

PDL1

CD40

CD137L

OX40L

CD28/CTLA-4

PD-1

CD40L

CD137

OX40

T cells

G. Kaplan
TUMOR BED

CD8⁺ T cell

CD8⁺ T cell

CD4⁺ T cell

CD4⁺ T cell

Tumor microenvironment

• Immunosuppressive factors
• Expression of inhibitory molecules
• Modulation of infiltrating leukocytes
• T cell “exhaustion” CTLA-4, PD-1
• Loss of antigen and HLA molecules

DC GENERATE ANTI-TUMOR IMMUNITY

TUMOR BED

Tumor microenvironment

IMMUNE ESCAPE
STIMULATING THE IMMUNE SYSTEM

- Adjuvants that activate DCs
- Tumor antigens
- Enhance T cell activation
- Trafficking of T cells to tumor site
- Reverse inhibition in tumor microenvironment
STIMULATING THE IMMUNE SYSTEM

Tumor bed

- Adjuvants that activate DC
  - TLR agonists
  - Anti CD40
  - Anti-DEC-205 + antigen
  - Radiation Therapy
  - Chemotherapy

- Enhance T cell activation
  - anti-CTLA-4 (IPILIMUMAB)
  - anti-CD137, OX40

- Trafficking of T cells to tumor site
  - T cell receptor transfection
  - Adoptive T cell therapy

- Reverse inhibition in tumor microenvironment
  - anti-PD-1/PDL-1
  - anti-TGF beta

Tumor bed
DC-BASED IMMUNOTHERAPY

- Antigen formulation
- Cell numbers
- Route of injection
- Combination therapies

ADJUVANTS

- Anti-DC antibodies fused to antigens
- Bacterial/viral vectors
- TLR stimulus
DC related therapeutics for cancer

• Vaccinate with DCs exposed to cancer proteins
  – Provenge for prostate cancer

• Target DCs with fusion proteins that contain tumor antigen and bind DCs
  – Recent studies in mice suggest targeting tumor antigens may enhance vaccination
Sipuleucel-T: PBMCs containing APCs, activated ex vivo with a recombinant fusion protein of a prostate antigen, prostatic acid phosphatase, fused to granulocyte–macrophage colony-stimulating factor.

22% reduction in risk of death compared with placebo group (hazard ratio, 0.78; 95% confidence interval [CI], 0.61 to 0.98; P=0.03).

4.1-month improvement in median survival
Current state— DC as cancer vaccine

• FDA recently approved the first DC vaccine clinical use – prostate cancer
  – Increasing median survival by 4.1 months
• Over 100 clinical trials have been designed
DC related therapeutics for viral infections

- Vaccinate with DCs exposed to HIV proteins
  - Reduce viral burden
  - As therapeutic vaccine enhanced CTL frequency, and decreased viral load after an STI (Garcia-Sastre Science Translational Medicine 2012)
DENDRITIC CELL VACCINES: RESULTS

DC exposed ex vivo to autologous inactivated HIV

Frequency of HIV specific T cells

Change in viral load set point

García-Sastre Science
Translational Medicine 2012
How Ralph Steinman Raced to Develop a Cancer Vaccine--And Save His Life

When Ralph M. Steinman developed pancreatic cancer, he put his own theories about cancer and the immune system to the test. They kept him alive longer than expected—but three days short of learning he had won the Nobel Prize.

By Katherine Harmon | Tuesday, December 20, 2011
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• Ralph Steinman
TARGETING DENDRITIC CELLS IN VIVO

- Peptides, whole proteins
- Tumor cells/lysates
- Anti-DC antibodies fused to antigens

ADJUVANTS

ACTIVATION OF TOLL-LIKE RECEPTORS (TLR) BY MICROBIAL MOLECULES

CNRS, France

DC maturation
TLR AGONISTS ACTIVATE DC TO STIMULATE ANTI-TUMOR IMMUNITY

TLR Agonist

TLR7
Imiquimod
“Aldara”

Normal skin
Imiquimod

CD4+ T cell responses after 4 vaccinations

No Ag

NY-ESO-1 OLP

Adams et al., J Immunol 2008
DC related therapeutics

• Block signal 2 – CTLA4-Ig
  – Lupus – higher numbers of DC and elev IFN-a
  – Transplant

• Block signal 3
  – Anti IL-6
    • Rheumatoid Arthritis
  – Anti p40 (IL-12/23)
    • Psoriasis