Macrophages and dendritic cells

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Pro-inflammatory cytokines: IL-1, IL-6, TNF, IL-23, IL-27, IL-18, IL-12.

↓

Type II immune IFN (IFN-γ)

Type I Interferon (IFN-α/β/ω/κ)

Conventional Dendritic Cells (IL-12→IFN-γ)

Plasmacytoid precursor Dendritic Cells (Type I Interferon-producing cells)

Antigen presentation, Co-stimulation, Immunomodulatory Cytokines

Type I Interferon (IFN-α/β/ω/κ)
Dendritic cells

- First identified in the epidermis (P. Langerhans, 1868)

- Characterized in lymphoid tissue (R. Steinman, 1973), and subsequently described in most organs; identified as the most efficient antigen-presenting cells.

- Methods to generate large numbers of dendritic cells in vitro (C. Caux, 1992; A. Lanzavecchia, 1994)

- Dendritic cells are «sentinels» controlling the immune response through highly-efficient antigen capture, processing, and presentation to antigen-specific CD4 and CD8 T cells.
Dendritic cells have specialized mechanisms for antigen-uptake

**Phagocytosis**
- **Bacteria**
  - Complement receptors (CR2)
  - Fc receptors (FcRII)
  - Mannose receptor
- **Zymozan**
- **Parasites**
- **Opsonized particles**
- **Apoptotic cells**
- **CD36, v3, v5?**

**Clathrin-dependent endocytosis**
- **HIV virus**
- **Desialylated IgG**
- **Hsp70/peptide**
- **Mannosylated compounds**
- **Immune complexes**
- **Lipoarabinomannan**
- **Fc receptors (FcRII, FdRI)**
- **Mannose receptor**
- **HSP receptor?**
- **CD4 and chemokine receptors?**
- **ILT3?**

**Macropinocytosis**
- **Soluble compounds**
Pathways of antigen processing and presentation by DC

• Endogenous antigen

• Exogenous antigen
In situ localization of human DC populations

Langerhans cells
(Langerin+ cells in skin epidermis)

Dermal DCs (interstitial DCs)
(CD1a+ in skin dermis)

Activated DCs (interdigitating DCs)
(DC-LAMP+ in T zone)

Plasmacytoid DCs
(CD123+ in T zone)

skin

tonsils
DC trafficking during pathogen invasion

1. Immature DC
2. DC recruitment
3. Antigen uptake
4. DC Activation and Emigration
5. T cell priming

Blood DC precursor

Pathogens

Tissue

Immature DC

Blood

CD34 Progenitors

Bone marrow

Mature DC

Lymphnode
DENDRITIC CELL ADOPTIVE THERAPY (VACCINATION)

Variables in DC adoptive therapy

- Antigen loading
- Origin and type of DC
- Maturation/activation of DC
- Routes of injection
# Dendritic cells are highly heterogeneous

<table>
<thead>
<tr>
<th>DC type</th>
<th>Tissue location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Langerhans cells</td>
<td>epidermis, stratified epithelia</td>
</tr>
<tr>
<td>Interstitial DC</td>
<td>dermis / lamina propria, blood, lymphoid tissue, organs</td>
</tr>
<tr>
<td>Interdigitating DC</td>
<td>T-zone lymphoid tissue, thymus</td>
</tr>
<tr>
<td>Veiled cells</td>
<td>lymph</td>
</tr>
<tr>
<td>Plasmacytoid DC (type-I IFN+++)</td>
<td>blood, lymphoid tissue</td>
</tr>
</tbody>
</table>
Monocyte-derived DC

Plasmacytoid DC

Precursor

Plasmacytoid DC

Monocyte

Modified from
Shortman and Liu 2002
Nature Review Immunology
Sources of DC in Clinical Trials

- Purified from Peripheral Blood
- CD34+ cell-derived (GM-CSF + TNF)
- Monocyte-derived (GM-CSF + IL-4)
Two pathways of dendritic cell development from CD34 hematopoietic progenitors

**DENDROCYTES: INTERSTITIAL TYPE DENDRITIC CELLS**
- CD14+, CD1a+, CD83+, CD86+
- BG+, Lag+, Langerin+, E-Cad+, CD68-, Fact. XIIIa-

**LANGERHANS CELLS: EPITHELIAL TYPE DENDRITIC CELLS**
- CD14+, CD1a+, CD83+, CD86+
- BG+, Lag+, Langerin+, E-Cad+, CD68+, Fact. XIIIa+

**TISSUE MACROPHAGES**
- CD14+, CD1a+, CD83+, CD86+
In vitro development of dendritic cells from CD34 hematopoietic progenitors or from monocytes.

**DENDROCYTES: INTERSTITIAL TYPE DENDRITIC CELLS**

- **CD34+**
- **GM-CSF + TNF**

**DENDROCYTES: INTERSTITIAL TYPE DENDRITIC CELLS**

- **CD14+**
- **GM-CSF + IL-4**
- **M-CSF**

**TISSUE MACROPHAGES**

- **CD14+**
- **GM-CSF + IL-4**
- **M-CSF**

**DENDROCYTES: INTERSTITIAL TYPE DENDRITIC CELLS**

- **CD1a+**
- **GM-CSF + IL-4**
- **M-CSF**

**LANGERHANS CELLS: EPITHELIAL TYPE DENDRITIC CELLS**

- **CD1a+**
- **GM-CSF + IL-4**
- **M-CSF**

**DAY 0**

- **BLOOD MONOCYTES**
- **CD14+**
- **GM-CSF + IL-4**
- **M-CSF**

**DAY 5**

- **PROGENITOR CELLS**
- **CD34+**
- **GM-CSF + TNF**

**DAY 14**

- **TISSUE MACROPHAGES**
- **DENDROCYTES: INTERSTITIAL TYPE DENDRITIC CELLS**
- **LANGERHANS CELLS: EPITHELIAL TYPE DENDRITIC CELLS**
In vitro development of Langerhans cells

**DENDROCYTES:**
INTERSTITIAL TYPE
DENDRITIC CELLS

**LANGERHANS CELLS:**
EPITHELIAL TYPE
DENDRITIC CELLS

**BLOOD MONOCYTES**

- CD14+
- GM-CSF + IL-4

**PROGENITOR CELLS**

- CD34+
- GM-CSF + TNF

- GM-CSF + IL-4 + TGFβ or GM-CSF + IL15

**CD14+**

- GM-CSF + TNF

**CD1a+**

- GM-CSF + TNF

**DENDROCYTES:**
INTERSTITIAL TYPE
DENDRITIC CELLS

**LANGERHANS CELLS:**
EPITHELIAL TYPE
DENDRITIC CELLS
Interstitial-like DC

Langherans-like DC

Interstitital-like DC

Modified from: Bancherau and Palucka, 2005
Variables in DC adoptive therapy

- Antigen loading
- Origin and type of DC
- Maturation/activation of DC
- Routes of injection
IMMUNITY

TOLERANCE

Modified from
Shortman and Liu 2002
Nature Review Immunology
Toll-like receptors
Other receptors
Autocrine cytokines

Activated NK cells
Activated NKT cells
Activated T cells

Cytokines (e.g. IFN-γ)
Cell-surface molecules (e.g. CD40L)

Modified from Mellman I et al, Trends in Cell Biology 1998
Immature DC                          Intermediate DC            Mature/activated DC

Modified from Mellman I et al, Trends in Cell Biology 1998
Antigen uptake

Signal 1

TNFα, LPS

Immature

+ + Antigen presentation molecules
MHC class I and II, CD1

++ Receptors for antigen uptake
Mannose receptor, DEC-205, Fcγ receptors

++ Endocytotic activity

+/− Motility

+/− Adhesion molecules
CD11a,b,c, CD50, CD54, CD58

++ Costimulatory molecules
CD80/B7.1, CD86/B7.2, CD40

Signal 2

IL-10, VEGF

Mature/activated

+/− Secretions
IL-12, T cell attractant chemokine

− Leukocyte differentiation antigens
CD3, CD8, CD19, CD20, CD56

− CD83

+/−

Signal 3

Timmerman JM, Levy R.

Dendritic cell vaccines for cancer immunotherapy.

Maturation/Activation of DC in Clinical Trials

• Monocyte-conditioned medium
• TNF-$\alpha$, IL-1$\beta$, IL-6, PGE2
• Trance/RANKL, CD40L
Variables in DC adoptive therapy

- Antigen loading
- Origin and type of DC
- Maturation/activation of DC
- Routes of injection
Routes of injection of Dendritic Cells

Pro-inflammatory cytokine and chemokine cascade

Modified from Randolph et al. Nature Review Immunology 2005
DC adoptive therapy / vaccination in cancer patients is safe, often induces an immune response against tumor-associated antigens, and in a proportion of cases induces a lasting partial or rarely complete remission that has been correlated with the extent of the immune response generated.

Clinical outcome of cancer vaccines in patients with melanoma

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Total patients</th>
<th>Responding patients</th>
<th>Response rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide vaccines</td>
<td>410</td>
<td>11</td>
<td>2.7</td>
</tr>
<tr>
<td>Viral vectors</td>
<td>160</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Tumour cells</td>
<td>43</td>
<td>2</td>
<td>4.6</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>116</td>
<td>11</td>
<td>9.5</td>
</tr>
</tbody>
</table>

(Data taken from: Rosenberg et al, Nature Medicine, 2004)

From: Bancherau and Palucka, Nature Review Immunology, 2005)
**Ex Vivo**

- Tumor-DC fusion
- Apoptotic tumor cells
- Apoptotic bodies
- Exosomes

- Protein/peptides
- Heat Shock Proteins

- cDNA or mRNA

**In Vivo**

- Mileau of proinflammatory cytokines and chemokines with recruitment of other cells
- Migration to lymphoid organs and maturation

- Receptor-mediated internalization

- CD8+ T-cell

- CD4+ T-cell

- MHCI

- MHCII

- Immature DC

- Mature DC

- MHCI

- MHCII

- Proinflammatory Cytokines and Factors

DC surface molecules

DEC205
(interdigitating DC
In T cell area of LN)

DC-SIGN
(dermal DC)

……..

The antigen-coupled antibodies reach DC in all tissues, including LN, and are internalized, inducing Ag-presentation to T cells

Immature DC → TOLERANCE

+ anti-CD40
+ TLR-ligands

Mature DC → IMMUNITY,
TUMOR REJECTION


Plasmacytoid precursor dendritic cells or type I Interferon producing cells.

Type I IFN-producing cells (IPC) (Plasmacytoid pre-DC)

- IPC are the only cell type in human blood able to produce type IFN in response to viruses
- IPC represent 1/500 to 1/200 of PBMC
- IPC are MHC class II positive but distinct in functions and morphology from Dendritic Cells
- IPC are very poor APC but are required for NK cell-mediated killing of virus-infected cells
- (1978-1986)
Characteristics of human myeloid DC and plasmacytoid precursor DC

**Conventional dendritic cells**
- **IL-12** in response to Gram + and - bacteria, intracellular parasites
- **IFN-α/β** secretion in response to polyI:C but not to most viruses

**Plasmacytoid precursor dendritic cells**
- (Liu, Briere, Colonna, 1999)
- Type I IFN producing cells (70-80s)
- High titer of **IFN-α** in response to viruses and certain CpG
- Low **IL-12** secretion

**TLR**
- **2±3+ 4±7±8+ 9-10-**
- **TLR** **2- 3-4- 7+ 8- 9+ (10+)**
The Toll-like (TLR) / IL-1 Receptor Family

Sequence variants in TLR4 and TLR1/6/10 have been associated with prostate cancer risk.

Nucleic acid ligands
- ssRNA
- R848
- Imiquimod
- dsRNA
- CpG DNA
- R848
- Cpg DNA

Protein ligands
- Uropathog. Bacteria Apicomplexan profilins
- flagellin
- LPS
- TLR11
- IL-1, IL-18
- IL-1R family
- MyD88
- TIRAP
- TRIF
- MD-2
- TRAM
- TLR1
- TLR2
- TLR3
- TLR4
- TLR5
- TLR6
- TLR7
- TLR8
- TLR9
- TLR10
- TLR11

Lipid ligands
- diacyl lipopeptides
- triacyl lipopeptides
- ?

Humans, Rat, Not Mouse

pH 5.5-6.5

Sequence variants in TLR4 and TLR1/6/10 have been associated with prostate cancer risk.

The Toll-like (TLR) / IL-1 Receptor Family
Human dendritic cell subsets

Coktail Fitc:
CD1a, 3, 14, 16, 19
CD4-PE Cy5
CD11c PE

Plasmacytoid DC
ILT3+ ILT1-
BDCA-2

Myeloid DC
ILT3+ ILT1+
BDCA-1=CD1c
Infected or inflammed tissues

Plasmacytoid Dentritic Cells

Afferent lymphacytic vessels

Peripheral tissues

Bone marrow

Infected or inflammed tissues


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Plasmacytoid Dendritic Cells

- pDC are very efficient producers of type I Interferon (IFN-a/b) and, in the Mouse but not in Humans, also produce IL-12.

- pDC are the main but not the only producer of type I IFN in most virus infections.

- By producing IFN and other cytokines, pDC are important players in the activation of innate resistance and inflammation and in the interface between innate and adaptive resistance.

- pDC are poor antigen presenting cells for T cells and are able to induce proliferation of pre-activated rather than naïve T cells.

- There are several reports that in vitro pDC may be tolerogenic and pDC have been shown to play an important role in regulating the response against harmless antigens in lung, liver, and gut (oral tolerance).

- By producing IFN and IL-6, pDC enhance B cell differentiation into plasma cells and may be involved in autoimmune diseases such as SLE and psoriasis.
Deficiency in pro-inflammatory genes results in resistance to carcinogenesis:
- IL-1, MIF, CSF-1, TNF, STAT3
- Anti-inflammatory cytokines prevent carcinogenesis: IL-10
- T regulatory cells may prevent H. hepaticus induced colon carcinogenesis by producing IL-10

Pro-inflammatory cytokines (e.g. IL-12, IL-18, IFN-γ, IFN-α, TNF, IL-2, IL-15) and ELR(-) interferon-inducible chemokines (IP-10, Mig, I-TAC) have anti-tumor and anti-angiogenesis effects.

TLR-ligands have anti-tumor activity that is enhanced by antagonism of IL-10.

Mice deficient for IFN-γ, IL-12, or IFN-α have increased incidence of spontaneous or carcinogenesis induced tumors.

The inflammatory and immune responses select tumors that have escape mechanisms and resist these responses.

CD1a⁺ DC in breast cancer are in direct contact with tumor cells

(≈30% positive tumors)

DC-LAMP⁺ and CD40⁺ DC in breast cancer are clustered in peri-tumoral lymphoid-like structures

(≈50% positive tumors)

255 patients with invasive non-metastatic breast cancer treated at Centre Leon Berard, Lyon, in 1996-1997

Tumor-infiltrating Dendritic Cells: Friends or Foes?

CD123+DC (and BDCA-2+) plasmacytoid DC) infiltrate human breast tumors in 13% of the patients. The presence of pDC in the primary tumor strongly correlates with poor prognosis.
Mouse TIDC are anergic but respond to select TLR ligands when IL-10 is inhibited

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**IL-12p40**

- **IFNγ**
  - none
  - anti-IL10R

- **TNFα**
  - none
  - 1668 CpG
  - R848
  - LPS + IFN-γ + aCD40
  - Poly I:C

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**IL-12p70**

- **IFNγ**
  - none
  - auto-IL10R

- **TNFα**
  - none
  - 1668 CpG
  - R848
  - LPS + IFN-γ + aCD40
  - Poly I:C
Interleukin-10 plays an important role in the immunosuppressive tumor environment.
Alternative Macrophage Activation

Tumor Associated Macrophages (TAM)
[Tumor Infiltrating Dendritic Cells (TIDC)]

- IFN-γ
- TLR agonists
- IL-4
- IL-13
- IL-10
- IL-12
- iNOS
- NO
- (IDO)
- TNF
- TGF-β
- (IL-12)
- Low Class II
- Arginases
- IL-1ra

M1

M2

Membrane receptors
- Scavenger receptor A
- Scavenger receptor B
- CD163
- MR
- TLR2, TLR4
- FcγRIIa, IL-1b, IL-6
- (CD16, CD32, CD84)
- CD80, CD86
- TNF-α
- IL-1
- IL-6
- IL-12
- Type I IFN
- IL-1 R type I
- Cytokine receptors
- Decoy IL-1 R type II

Cytokines
- IL-1 ra
- IL-10
- IL-12
- IL-10
- IL-12

Chemokines
- CCL17, CCL22
- CCL24
- CCL16
- CCL18
- CCL11
- CCL4, CCL5
- CXCL9, CXCL10, CXCL11

Chemokine receptors
- CCR2
- CXCR1, CXCR2
- CCR7
- Effector molecules
- Arginase

Arginases
- IFN-γ and LPS
- IL-10
- IL-4 and IL-13
- IL-4 and IL-13, IL-10

Mantovani et al, 2002

TRENDS in Immunology
Is it possible to induce an immune response to the tumor own antigens by removing immuno-suppression and providing an inflammatory stimulus?

TSA tumors (>5mm) treated with adenovirus vector expressing LEC/CCL16, intratumoral CpG, and systemic anti-IL-10R.

(similar results for TSA, 4T1, MC38 and for inhibition of spontaneous metastases of 4T1)
CCL16 + CpG + anti-IL-10R treatment induces a rapid hemorrhagic tumor necrosis that is dependent on TNF, IL-12, and CD40.
I. Inflammatory Hemorrhagic Necrosis 6-16 h p.t.

- M2 macrophages
- IL-10
- Arginase

CD4+ CD8+

II. Tumor Ag-specific Effector CD8+ T cells

- Draining LN
- CD25+ CD4+ TREG

- Tumor Ag-specific regulatory CD8+ T cells

CD40/CD40L

DC migration to draining LN 0-6 h p.t.

CpG-OGN

Anti-IL-10R (anti-CD25)

Nitric Oxide

TNF

IL-12

IFN-γ

CD40/CD40L

TNF

CCR7/CCR7L
Alain Vicari  
Christophe Dercamp  
Christophe Caux  
Francine Brière  
Serge Lebecque

LIR, Schering-Plough Research Institute  
Dardilly, France

Cristiana Guiducci  
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Milano, Italy

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