Dendritic cells

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The pathogen-derived antigens need to be transported. Adaptive immune response needs to be initiated.
Immunity and Anti-immunity – Host Vs Pathogen

- Innate immune cells
  - Phagocytosis
  - Neutrophils
  - NET

- Adaptive immunity
  - CD4 T
  - Th1
  - Th2
  - Th17
  - Tregs

- Anti-microbial responses
- Priming adaptive immune response

Pathogens
- Bacteria, virus, fungi
Dendritic cells - Discovery

1868  Paul Langerhans => Branched skin cells at epidemis resembling neurons
Dendritic cells - Discovery

1868  Paul Langerhans
      => Branched skin cells at epidemis resembling neurons

1973  Ralph Steinman
      => Dendritic cells from the spleen of mice
One of the first views of DCs in mouse spleen

Dendritic cells: Major professional antigen presenting cells

Critical for primary immune response

Large array of endocytic receptors (not B cells)

Large array of antigen presenting and co-stimulatory molecules (not MØ)

Ability to migrate to T cell areas for presentation (not other APCs)
Some features of dendritic cells

Adhesion and co-stimulation
ICAM 1,3; B7-1, B7-2; CD58/LFA3

Secretion
IL-12, IL-10, TNF, chemokines

High levels of Ag presenting molecules
HLA I, II; CD1

Receptors for Ag uptake
MMR, DC205, Langerin, integrins, FcγR TLR, CR

Signaling of DC
TNF-R, CD40, TLR
Cytokine R: GM-CSF, IL-4, IL1, TGF-b, IL-10

Receptors for pathogens
CD4, DC-SIGN, chemokine receptors
Dendritic cells in health and disease
Ontogeny of dendritic cells
Origin and development of dendritic cells

MP: Myeloid progenitor, MDP: monocyte dendritic cell progenitor, CDP: committed DC progenitor

Flt3L: fms-like tyrosine kinase 3 ligand

Liu & Nussenzweig, Immunol Rev 2010
Subsets of dendritic cells
Dendritic cell subsets in health and disease

Epidermal Langerhans cells
- C-type lectin
- Langerin
- CD11c
- CD1a
- E-cadherin
- TLRs: 1, 2, 3, 6, (7), (10)

Dermal Interstitial DCs
- DC-SIGN
- Mannose Receptor
- CD11c
- CD1a/CD14
- CD11b
- CD36
- Factor XIIIa
- TLRs: 1, 2, 3, 4, 5, 6, 7, 8

Blood Myeloid DCs
- (DC-SIGN)
- Mannose Receptor
- CD11c
- TLRs: 1, 2, 3, 4, 5, 6, (7), 8, 10

Blood Plasmacytoid DCs
- BDC-2
- CD123
- ILT7
- TLRs: 1, 6, 7, 9, 10
Human dendritic cell deficiency

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DC deficiency syndrome</th>
<th>IRF8 mutation (K108E)</th>
<th>IRF8 mutation (T80A)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>DCML deficiency</td>
<td></td>
<td></td>
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<tr>
<td>CD123⁺ plasmacytoid DCs</td>
<td>Population absent</td>
<td>Population absent</td>
<td>Normal</td>
</tr>
<tr>
<td>CD11c⁺ myeloid DCs</td>
<td>Population absent</td>
<td>Population absent</td>
<td>Normal</td>
</tr>
<tr>
<td>CD1c⁺ myeloid DCs</td>
<td>Population absent</td>
<td>Population absent</td>
<td>Normal</td>
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<tr>
<td>CD141⁺ myeloid DCs</td>
<td>Population absent</td>
<td>Population absent</td>
<td>Normal</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Severely reduced in number</td>
<td>Severely reduced in number</td>
<td>Normal</td>
</tr>
<tr>
<td>B cells</td>
<td>Reduced in number</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Natural killer cells</td>
<td>Reduced in number</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>T cells</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Defective cytokines</td>
<td>IL-12, IFNγ, IL-6, TNF</td>
<td>IL-12, IFNγ, IL-6, TNF</td>
<td>IL-12</td>
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<tr>
<td>Myeloproliferation</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Numbers of CD34⁺ cells in the periphery</td>
<td>Increased</td>
<td>Increased</td>
<td>Not determined</td>
</tr>
<tr>
<td>Serum levels of FLT3L</td>
<td>50–100-fold elevation</td>
<td>2–3-fold elevation</td>
<td>Normal</td>
</tr>
<tr>
<td>Regulatory T cells</td>
<td>Reduced in number</td>
<td>Reduced in number</td>
<td>Not determined</td>
</tr>
<tr>
<td>GMPs and MLPs</td>
<td>GMPs reduced in number; MLPs absent</td>
<td>Populations expanded</td>
<td>Not determined</td>
</tr>
<tr>
<td>Age of patient at diagnosis</td>
<td>7–60 years</td>
<td>&lt;3 months</td>
<td>12–15 months</td>
</tr>
<tr>
<td>Inheritance pattern</td>
<td>Autosomal dominant or de novo mutation</td>
<td>Autosomal recessive</td>
<td>Autosomal dominant or de novo mutation</td>
</tr>
</tbody>
</table>

DC, dendritic cell; DCML, DC, monocyte, B and NK lymphoid; FLT3L, FMS-related tyrosine kinase 3 ligand; GMPs, granulocyte-macrophage progenitors; HPV, human papilloma virus; IFNγ, interferon-γ; IL, interleukin; IRF8, interferon regulatory factor 8; MLPs, multi-lymphoid progenitors; TNF, tumour necrosis factor.

Collin et al., Nature Rev Immunol 2011
Purification / preparation of dendritic cells

DCs – Low population

- <1% Spleenocytes
- <1% blood leukocytes
- <1% tissues (skin, lungs)

Obtaining DC

1992  J Banchereau et C Caux (Lyon)

- Bone marrow cells → DC human

1992  R Steinman (NY)
      K Inaba (Kyoto)

- Splenic cells → DC murine

1994  A Lanzavecchia (Bellinzona, Suisse)

- Blood monocytes → DC or macrophages
Host immune response to pathogens

- Pathogens
- Dendritic cell
- CD4+ Th cell
- B cell
- Antibodies
- Bacteria, fungi
- Humoral/antibody response
MHC Class II presentation by Dendritic cells

Invariant chain (Ii)
MHC class II compartment (MIIC)
Class II-associated Ii peptide (CLIP)

Neefjes et al 2011
Host immune response to pathogens

- Dendritic cell
- CD4+ Th cell
- B cell
- CD8+ Tc cell

Pathogens

Antibodies

Humoral/antibody response

Intracellular bacteria, virus

CTL response
MHC Class I presentation by Dendritic cells

TAP: transporter associated with antigen presentation,
ER: endoplasmic reticulum

Neefjes et al 2011
CD4+T cell immune response to pathogens

Antigen

Endosome/Lysosome

MHCII

APC

PRR
Airborne fungal spores

Up to $10^9$/m$^3$

A. fumigatus

Healthy

Immunocompromised

Elimination by phagocytosis

Invasive aspergillosis

Innate immune cell

SOIL
A. fumigatus sensing by innate cells – Role of Rodlet

Rodlet layer

Immune response – Inert

Immune response – Activated

Dormant conidia

Enlarged or Germinated conidia

Rodlet layer degraded

Aimanianda*, Bayry* et al Nature. 2009
A. fumigatus – structural organization of the conidia

Electron micrograph of fixed conidia

Courtesy: Beauvais A

Rodlet layer

Polysaccharides
Proteins
Lipids

Exposure of polysaccharides during germination

PAMPS

α-(1,3)-glucan
β-(1,3)glucan
Chitin
Galactomannan
General characteristics of the four main pattern recognition receptor families expressed by DCs. The most important effects of PRR triggering are given in italics. Abbreviations: PAMP, pathogen-associated molecular pattern; Ag, antigen; PGN, peptidoglycan
PRRS and DCs – 2011 Noble prize

Toll receptors

Chitin

Dendritic cells

PRRS and DCs – 2011 Noble prize
Changes during dendritic cell maturation

Immature DC

Mature DC
Changes during dendritic cell maturation

Immature DC
- High intracellular HLA II
- High endocytosis
- Low CD54, 58, 80, 86
- Low CD40
- Low CD83
- Low granule antigens

Mature DC
- High surface HLA II
- Low endocytosis
- High CD54, 58, 80, 86
- High CD40
- High CD83
- Production of inflammatory cytokines

Pathogens
Pro-inflammatory cytokines
T cells
Methodology

- PBMC
  - CD14 Monocytes
    - GM-CSF & IL-4
    - 5 days
  - MoDC
    - A. fumigatus (morphotypes)
      - Dormant, Enlarged, Germinated conidia
      - 48 hrs
      - DC responses
Enlarged and germinated conidia but not dormant conidia induce maturation and activation of human dendritic cells.
Migration of dendritic cells to draining lymph nodes
CD4+ T cell immune response to pathogens
Dendritic cells in health and disease
Regulation of Dendritic cell functions by immune compartment
Regulation of Dendritic cell functions by immune compartment

- Effector CD4+ T cells
- B cells / Immunoglobulins
- Cytokines
- Regulatory T cells
Regulation of Dendritic cell functions by immune compartment

- Effector CD4+ T cells
- B cells / Immunoglobulins
- Cytokines
- Regulatory T cells
T cells induce activation of dendritic cells

Co-stimulatory molecules

MFI

None  RO  RA

CD80

CD86

None  RO  RA
T cells induce activation of dendritic cells

Inflammatory cytokines

- TNF-α
- IL-6
- IL-8

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>RO</th>
<th>RA</th>
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<tbody>
<tr>
<td>TNF-α</td>
<td></td>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
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<td>300</td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td>0</td>
<td>300</td>
</tr>
</tbody>
</table>
Stimulation of dendritic cells by CD40L (CD154) on T cells leads to maturation

HLA-DR  CD11c  CD86  CD80  CD40

Anti-CD154/CD40L  Isotype Ctrl
Dendritic cells and regulatory T cells

CD4+CD25+ regulatory T cells in human circulation

• Represent ~2-4% of total PBMCs

• Express CD25

• FoxP3 (transcription factor forkhead)

• Intracellular CTLA-4 Expression

• Immunosuppressors
CD4+CD25+ regulatory T cells: Mechanisms of suppression

Vignali et al., 2008; Nat Rev Immunol
CD4+CD25+ regulatory T cells: Mechanisms of suppression

**a. Inhibitory cytokines**

- Membrane-tethered TGFβ
- IL-35
- IL-10

**b. Cytolysis**

- Granzyme A or granzyme B
- Perforin pore
- Apoptotic effector T cell

**c. Metabolic disruption**

- CD25
- IL-2
- cAMP
- ADAR

**d. Targeting dendritic cells**

- CTLA4
- CD80/CD86
- LAG3
- MHC class II

Death due to cytokine deprivation

Inhibition of DC maturation and function

Vignali et al., 2008; Nat Rev Immunol
CD4+CD25+ regulatory T cells inhibit TLR-mediated activation of human dendritic cells

Co-stimulatory molecules

CD80

CD86

MFI

None Treg RO RA

None Treg RO RA

J Immunol 2004
J Immunol 2007
Arthritis Rheum 2007
Nature 2008
Arthritis Rheum 2009
Nature Rev Rheumatol 2009
Am J Pathol 2009
Vaccine 2011
CD4+CD25+ regulatory T cells inhibit TLR-mediated activation of human dendritic cells

Inflammatory cytokines

**TNF-α**

**IL-6**

**IL-8**

J Immunol 2004
J Immunol 2007
Arthritis Rheum 2007
Nature 2008
Arthritis Rheum 2009
Nature Rev Rheumatol 2009
Am J Pathol 2009
Vaccine 2011
CTLA-4 is the major molecule implicated in the CD4+CD25+ regulatory T cell-mediated inhibition of dendritic cells.
CD4+CD25+ regulatory T cells inhibit T cell proliferation mediated by TLR-activated human dendritic cells

Bayry et al., J Immunol. 2007
CD4+CD25+ regulatory T cells inhibit DC-mediated IFN-γ responses
Regulation of Dendritic cell functions by immune compartment

- Effector CD4+ T cells
- B cells / Immunoglobulins
- Cytokines
- Regulatory T cells
Regulation of Dendritic cell functions by immune compartment

Type I interferon:
Promotion of activation of DC

Maddur et al., Arthritis Rheum 2010
Regulation of Dendritic cell functions by immune compartment

TGF-β: inhibit the DC maturation
Regulation of Dendritic cell functions by immune compartment

- Effector CD4+ T cells
- B cells / Immunoglobulins
- Cytokines
- Regulatory T cells
Regulatory B cells slow down the complete maturation of iDCs into mDCs.

B cell stimulation:
CD40L+CpG-ODN 2006

**Figure:**
- **HLA-DR**, **CD80**, and **CD86** expression levels are shown for different conditions: Ctrl, Mo+B+BCR, and Mo+B+BCR/CpG.
- The MFI (Mean Fluorescence Intensity) values are compared for HLA-DR, CD80, and CD86.

**Graph:**
- The bar chart displays the MFI values for each condition, with significant differences indicated by asterisks (*) for Mo+B+BCR/CpG compared to Ctrl and Mo+B+BCR.

**References:**
Activated B cells induce dendritic cell maturation and induce Th2 polarization by inducing OX-40 ligand

Maddur et al., Nature Commun 2014
Activated B cells induce dendritic cell maturation and induce Th2 polarization by inducing OX-40 ligand

Maddur et al., Nature Commun 2014
Regulation of Dendritic cell functions by immune compartment

- Effector CD4+ T cells
- B cells / Immunoglobulins
- IgG immunoglobulins at therapeutic high doses (IVIg)
- Cytokines
- Regulatory T cells
Regulation of dendritic cells by circulating antibodies: Intravenous Immunoglobulin (IVIg)

Intravenous Immunoglobulin (IVIg)

- Therapeutic preparation of pooled normal polyspecific human IgG obtained from large numbers of healthy donors

Contains

Antibodies to non-self (foreign antigens)
Antibodies to self antigens
Antibodies to antibodies (anti-ids)
Intravenous Immunoglobulin (IVIg)

Polyclonal IgG – plasma pool – thousands of healthy donors

Primary Immunodeficiencies
- CVID,
- XLA

Secondary Immunodeficiencies
- BM transplantation,
- Multiple myeloma

Autoimmune and inflammatory diseases
- Immune thrombocytopenic purpura (ITP)
- Guillain-Barré syndrome (GBS)
- Kawasaki disease (KD)
- CIDP
- Dermatomyositis (DM)
- Polymyositis (PM)
- Psoriasis
- Systemic lupus erythematosus (SLE)
- ANCA-associated vasculitis
- Myasthenia gravis (MG)
- Transplant rejection

Wide - divergent therapeutic applications
Regulation of maturation and function of dendritic cells by IVIg at therapeutic concentration

**CD1a**

*60 (80)*

*15 (21)*

**CD83**

*43 (39)*

*1 (27)*

**IL-12**

- **Ctl**
- **IVIg**
- **HSA**

**IL-10**

- **Ctl**
- **IVIg**
- **HSA**

**Thymidine Incorporation (x10⁴)**

- **Ctl**
- **IVIg**
- **HSA**

Contribution of DC to autoimmunity

- Genetic
- Epigenetic
- Environmental

- Cytokines/chemokines
- Ag presentation
- Co-stimulatory molecules
- Migratory properties

- Effector /pathogenic T cells
- Treg number and function
Dendritic cells prevent autoimmunity by expansion of regulatory T cells
Regulatory T cell expansion by dendritic cells

Transforming growth factor-β (TGF-β)
Cyclooxygenase-2 (COX-2)
Indoleamine 2,3-dioxygenase (IDO)

Inducible costimulatory ligand (ICOS-L)
CD252 (OX40L)

Programmed death ligands

Pallotta et al., Nat Immunol. 2011
Trinath et al., Blood 2013
Gopisetty et al., Immunology 2013
Fantini et al., Immunology 2004
Sakaguchi et al., Cell, 2008
Dejaco et al., Immunology, 2007
Regulatory T cell expansion by dendritic cells: Role of Programmed death ligands

% positive dendritic cells

Ctr Mtb Ctr Mtb Ctr Mtb

*= significant difference

PD-L2 PD-L1 PD-1

Trinath et al. J Infect Dis 2012
Regulatory T cell expansion by dendritic cells: Role of Programmed death ligands

% CD4+CD25+FoxP3+ T cells

Ct r Mtb Mtb+ anti-PD-L1

* *
Regulatory T cell expansion by dendritic cells: Role of PGE2

COX-2

β-actin

PGE2 (pg/ml)

Ctr  IVIg 10 mg  IVIg 15 mg  HSA

Ctr  IVIg 10 mg  IVIg 15 mg  HSA

Trinath et al Blood 2013
Regulatory T cell expansion by dendritic cells: Role of PGE2

Trinath et al. Blood 2013
Comparison of different cell populations for the induction of CD4+CD25+ FOXP3+ Treg's.

Differentiation of iTregs by dendritic cells

Banerjee D K et al. Blood 2006;108:2655-2661
Immune tolerance
Prevention of autoimmunity
Induction of Tregs
Maintenance/homeostasis of Tregs

Immature/tolerogenic DC

T and B cell response to pathogens
Clearance of pathogens
Anti-tumor immunity

Activated DC

Take home message
Take home message

Immune tolerance
Prevention of autoimmunity
Induction of Tregs
Maintenance/homeostasis of Tregs

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Activated DC

T and B cell response to pathogens
Clearance of pathogens
Anti-tumor immunity

Autoimmunity
Inflammatory diseases

Genetic
Epigenetic
Environmental
Thank you