Primer on Tumor Immunology and Biological Therapy
November 10, 2005
Hilton Alexandria Mark Center
Alexandria, VA

Macrophages and dendritic cells

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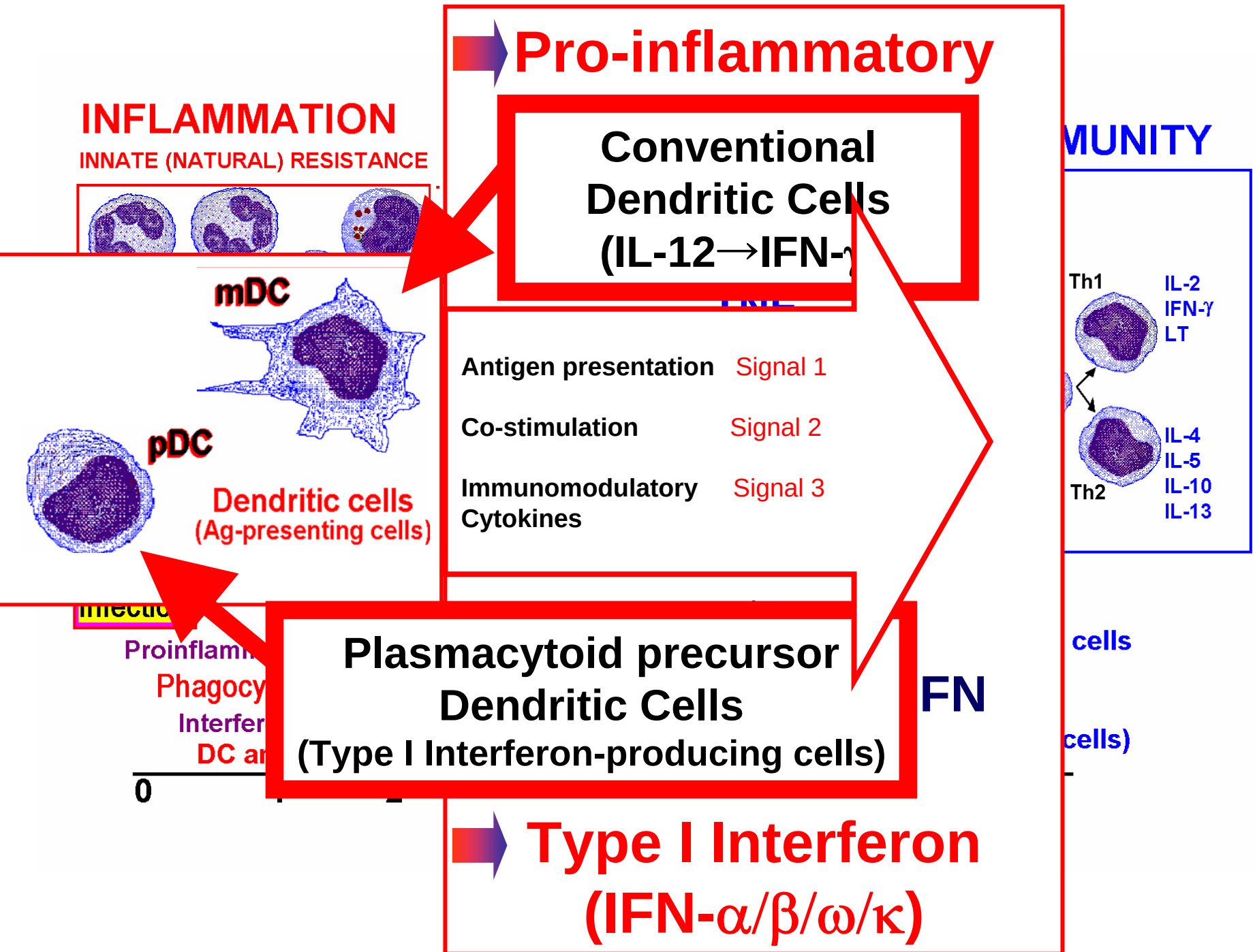
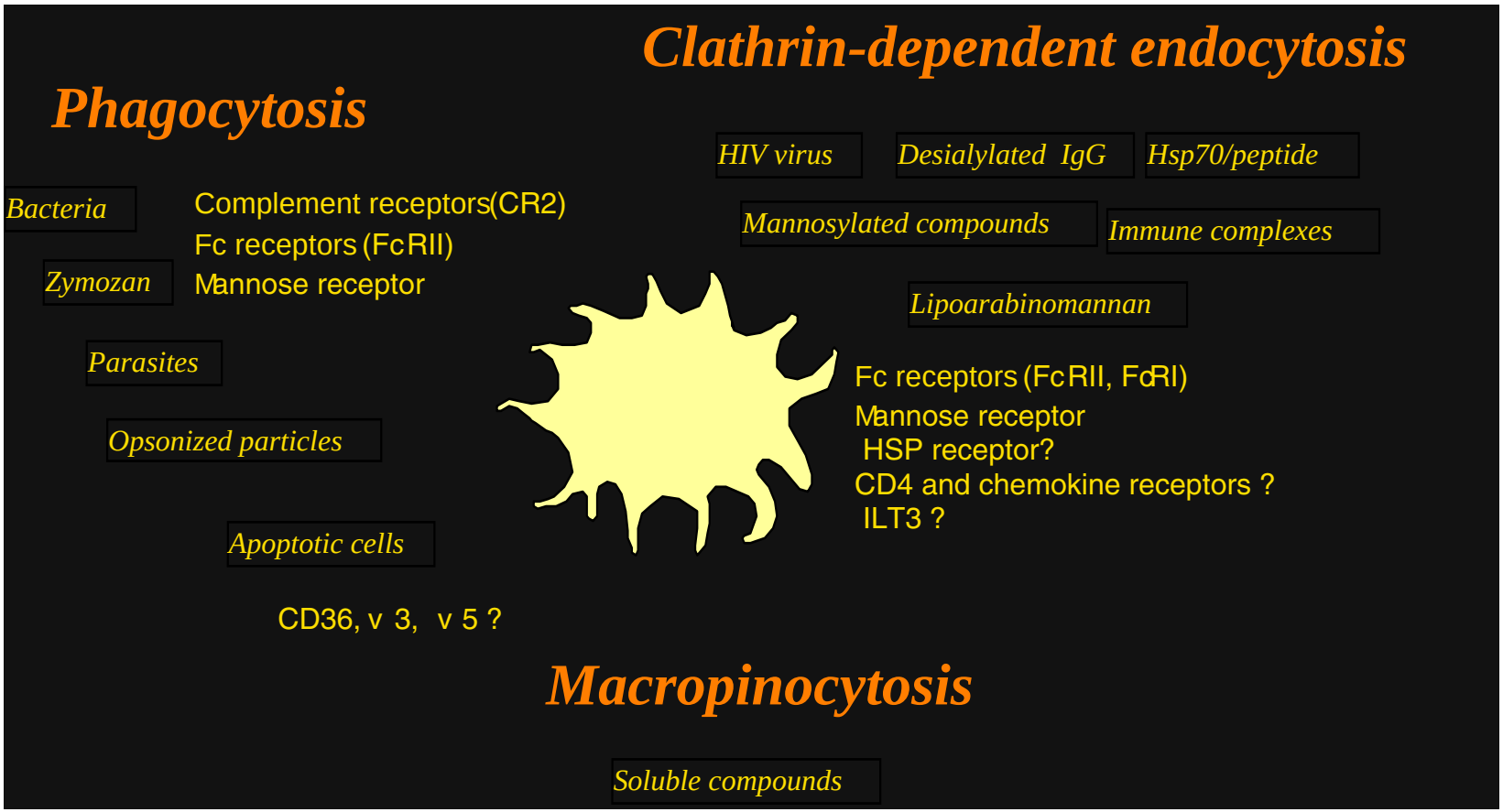

Pro-inflammatory
INFLAMMATION
INNATE (NATURAL) RESISTANCE
Conventional
Dendritic Cells
(IL-12→IFN-γ
MUNITY
mDC
pDC
Dendritic cells
(Ag-presenting cells)
Antigen presentation Signal 1
Co-stimulation Signal 2
Immunomodulatory Signal 3
Cytokines
Th1
IL-2
IFN-γ
LT
IL-4
IL-5
IL-10
IL-13
Th2
Proinflam
Phagocy
Interfer
DC ar
0
cells
cells)
FN
Plasmacytoid precursor
Dendritic Cells
(Type I Interferon-producing cells)
Type I Interferon
(IFN-α/β/ω/κ)

Dendritic cells

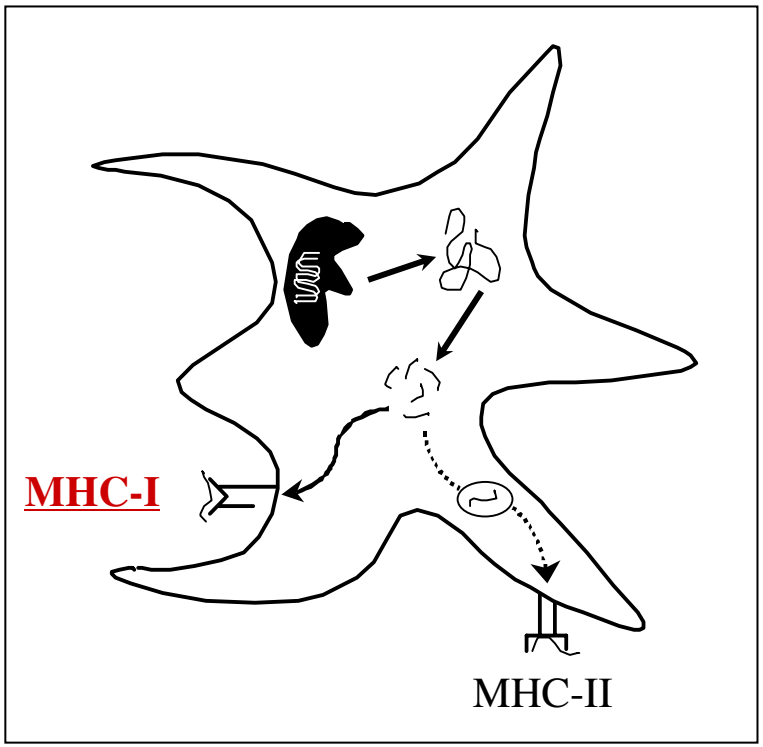
- First identified in the epidermis (*P. Langerhans, 1868*)
- Characterized in lymphoid tissue (*R. Steinman, 1973*), and subsequently described in most organs; identified as the most efficient antigen-presenting cells.
- Methods to generate large numbers of dendritic cells *in vitro* (*C. Caux, 1992; A. Lanzavecchia, 1994*)
- Dendritic cells are «sentinels» controling 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

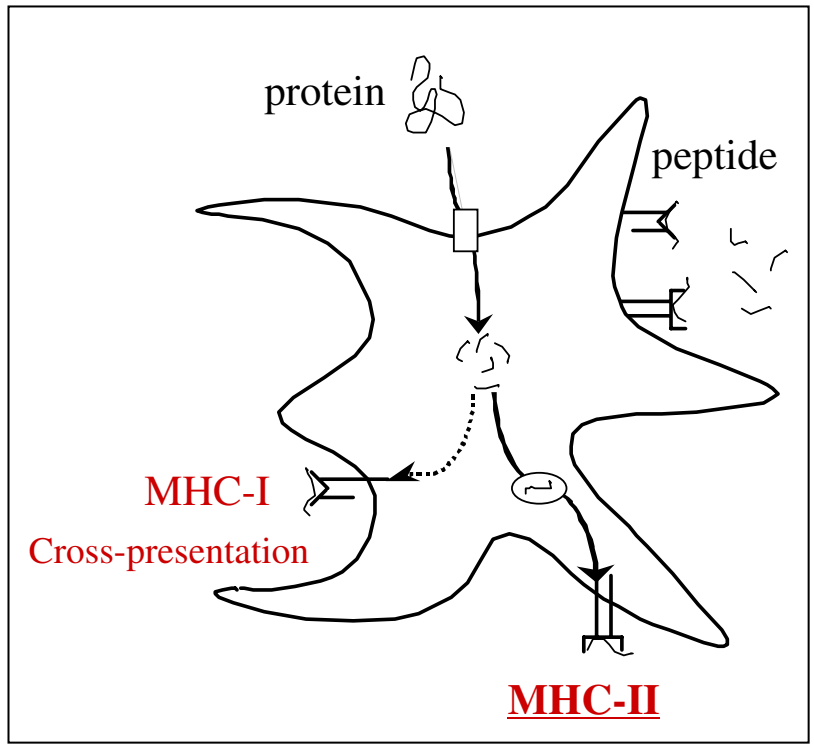


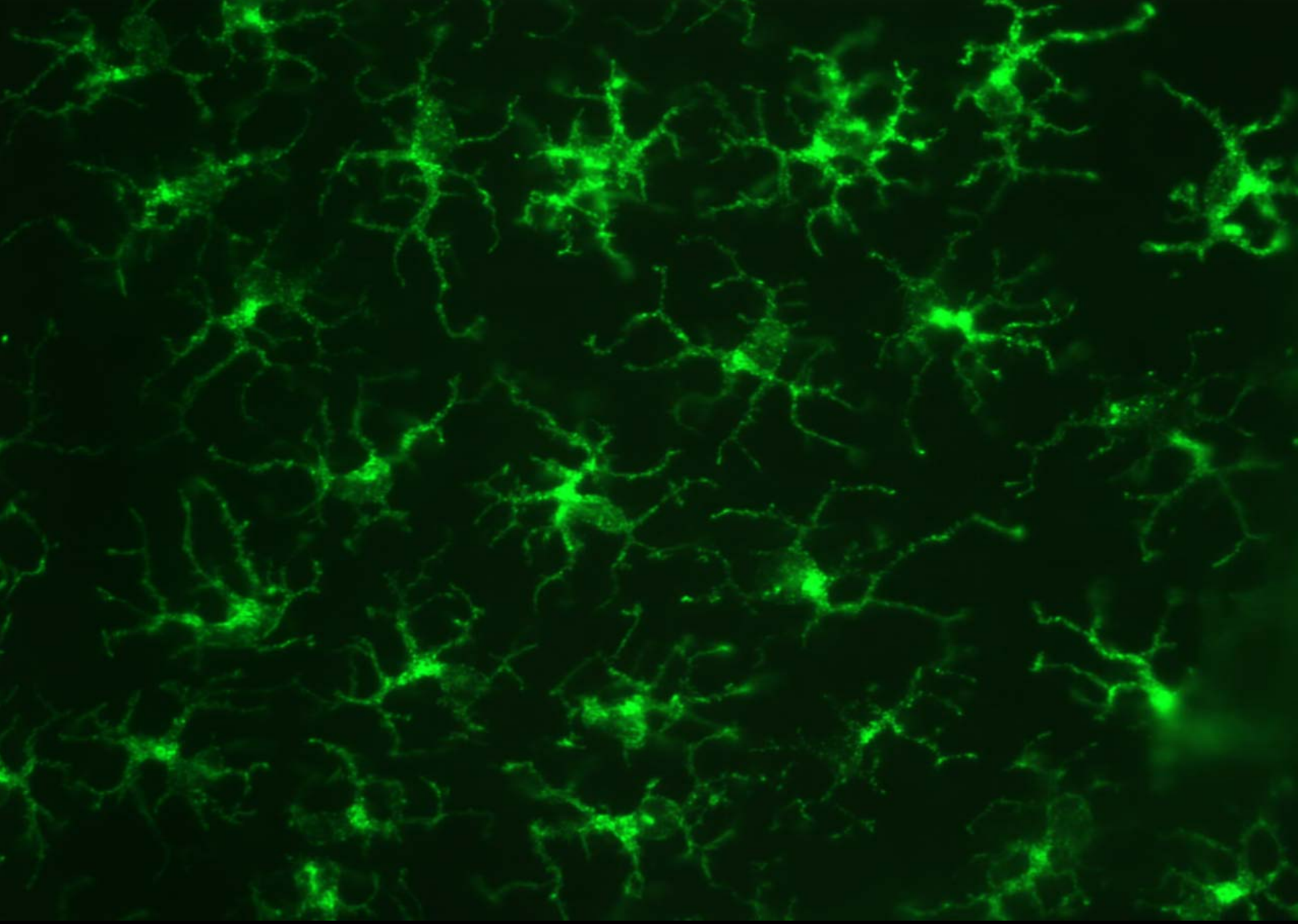
Pathways of antigen processing and presentation by DC

- **Endogenous antigen**



- **Exogenous antigen**

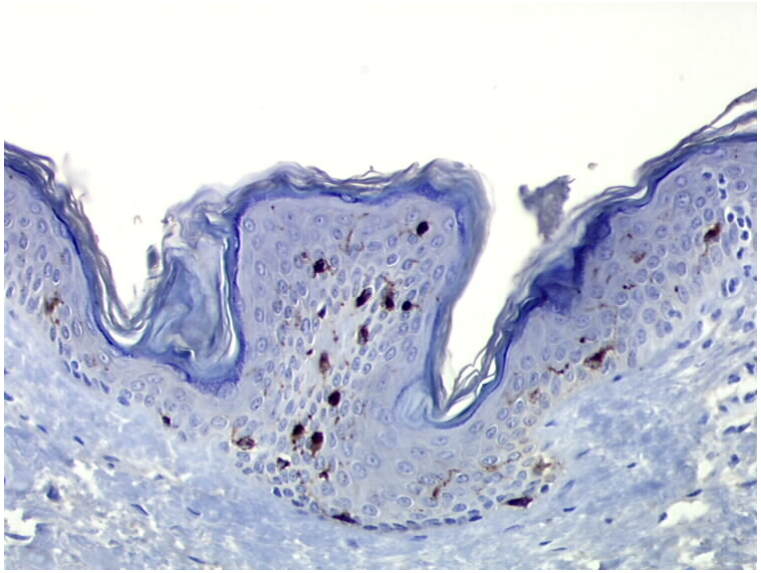




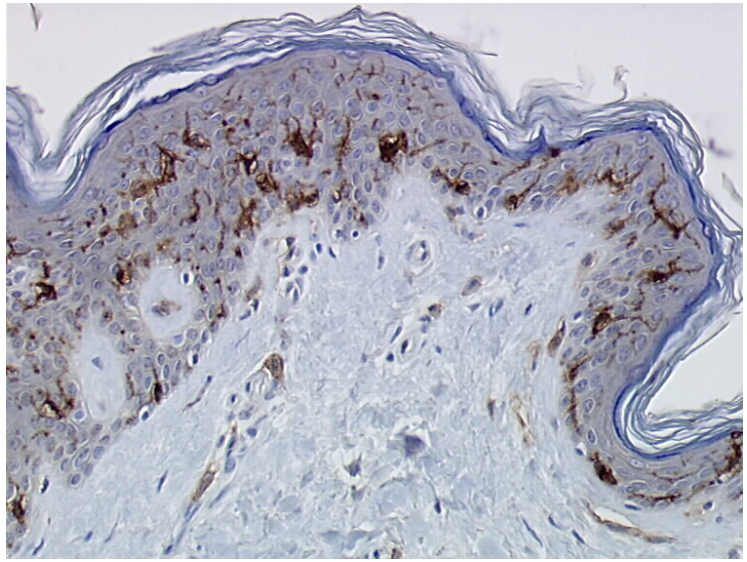
In situ localization of human DC populations

skin

Langerhans cells
(Langerin⁺ cells in skin epidermis)

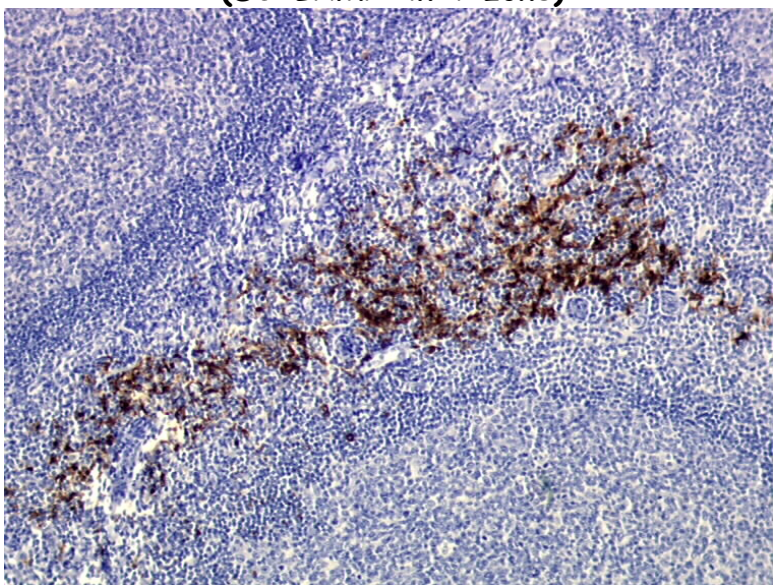


Dermal DCs (interstitial DCs)
(CD1a⁺ in skin dermis)

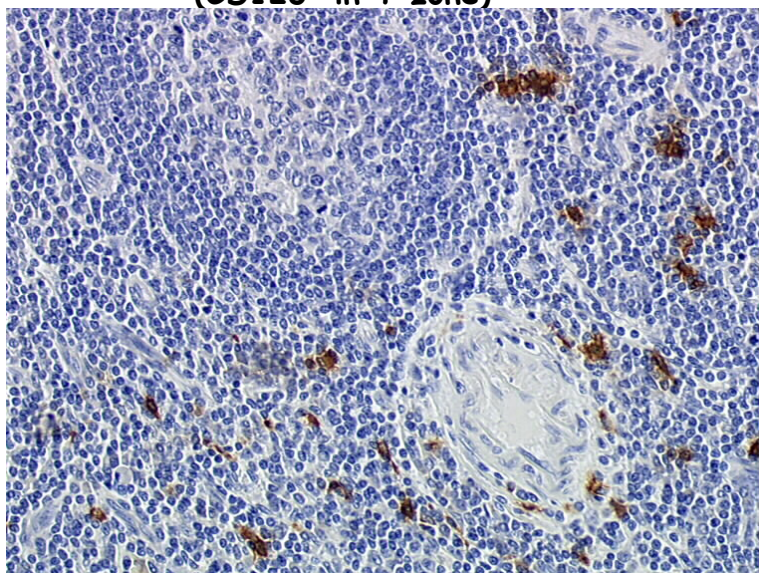


tonsils

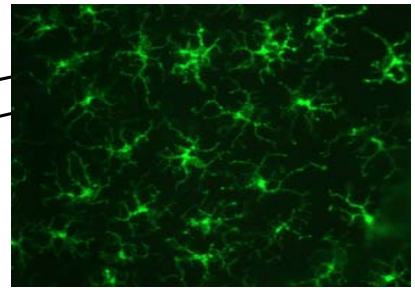
Activated DCs (interdigitating DCs)
(DC-LAMP⁺ in T zone)



Plasmacytoid DCs
(CD123⁺ in T zone)



DC trafficking during pathogen invasion



1 *Pathogens*

Immature DC

Tissue

DC

3 *Antigen uptake*

4 *DC Activation and Emigration*

Blood DC precursor

2 *DC recruitment*

DC

DC

DC

Lymph

Blood

CD34+ Progenitors

Bone marrow

5 *T cell priming*

DC

DC

DC

T

T

T

T

T

T

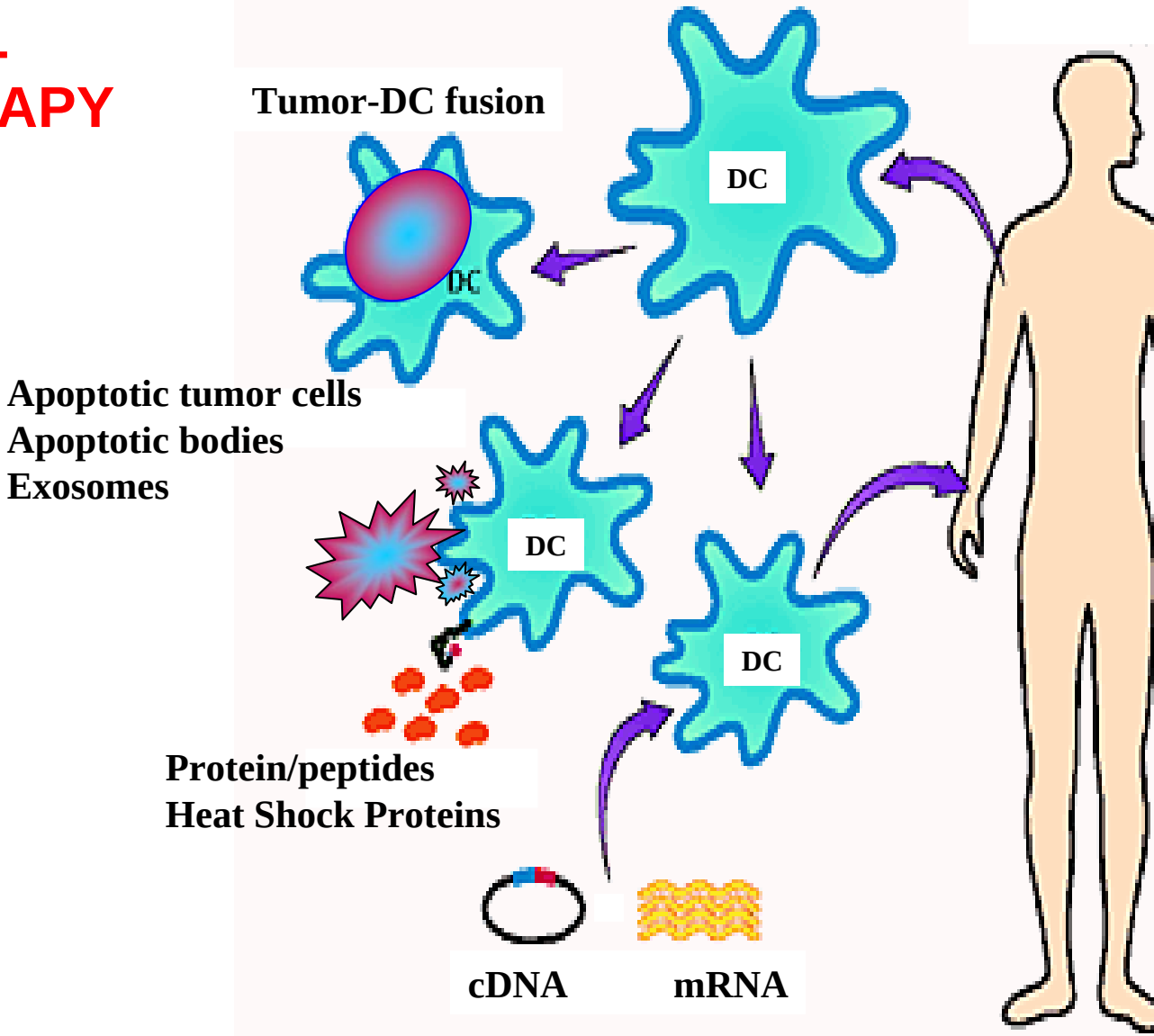
Mature DC

Lymphnode

Ex Vivo

DENDRITIC CELL ADOPTIVE THERAPY

(VACCINATION)



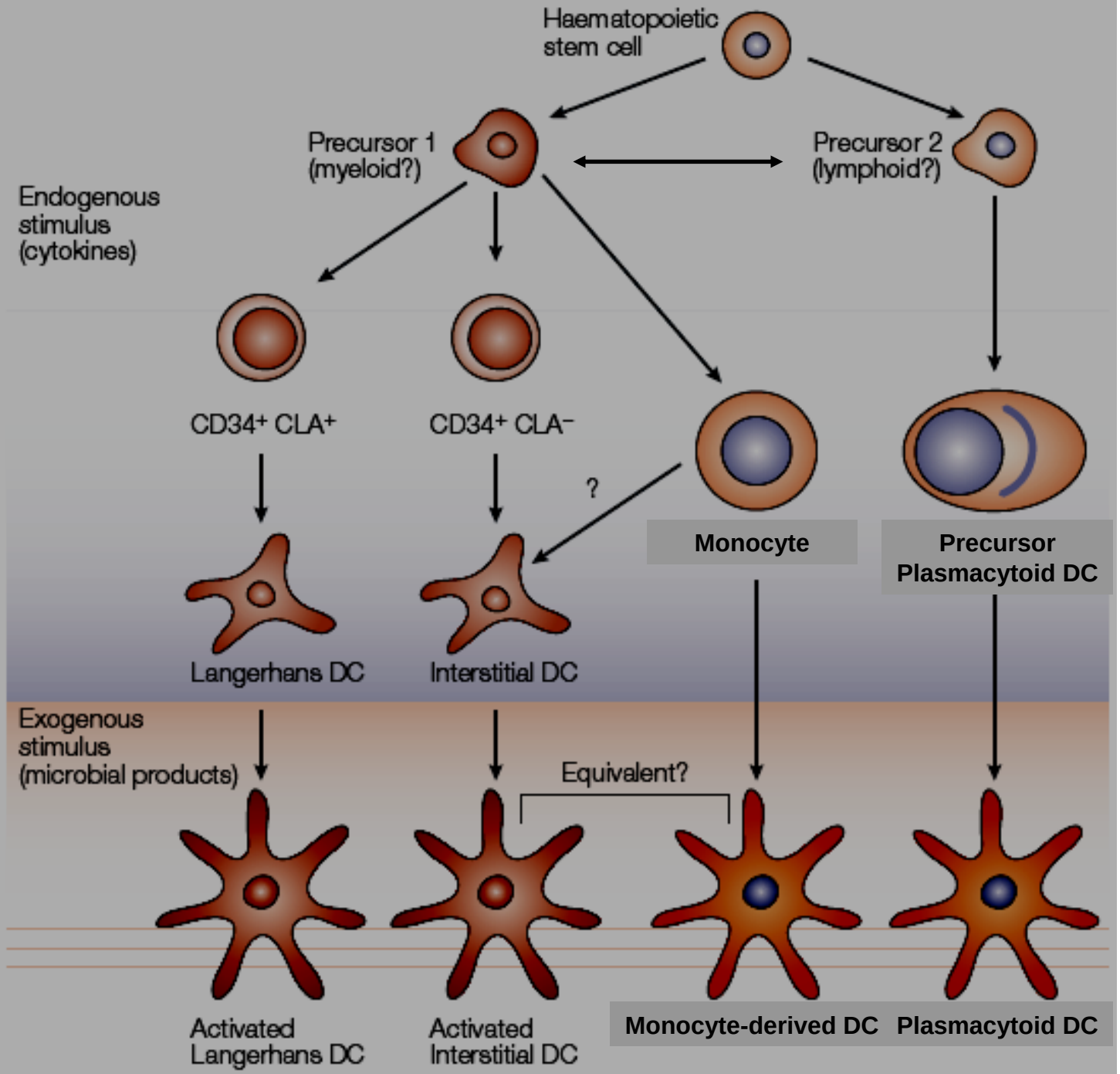
Biragyn & Kwak: Nature Med. 6:966 (2000) (modified)

Variables in DC adoptive therapy

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

Dendritic cells are highly heterogeneous

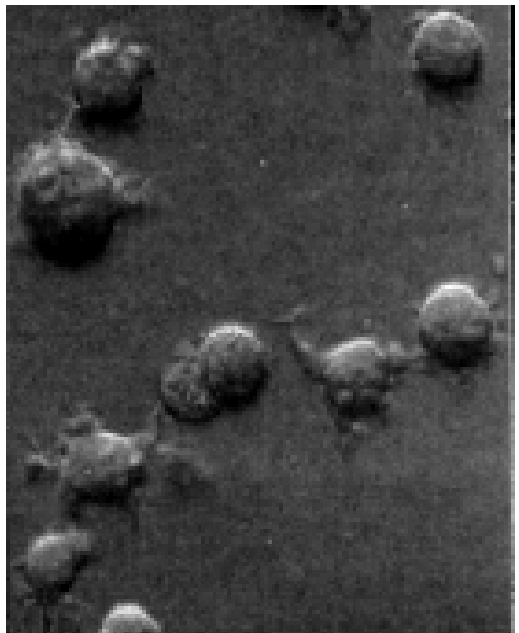
DC type	Tissue location
Langerhans cells	epidermis, stratified epithelia
Interstitial DC	dermis / lamina propria , blood, lymphoid tissue, organs
Interdigitating DC	T-zone lymphoid tissue, thymus
Veiled cells	lymph
Plasmacytoid DC (type-I IFN+++)	blood, lymphoid tissue

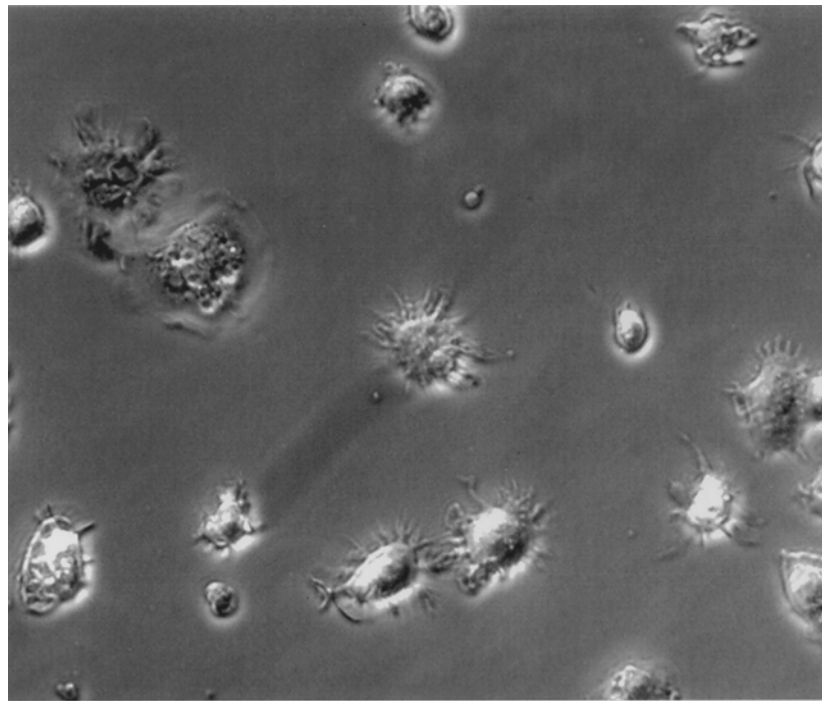


Modified from
Shortman and Liu 2002
Nature Review Immunology

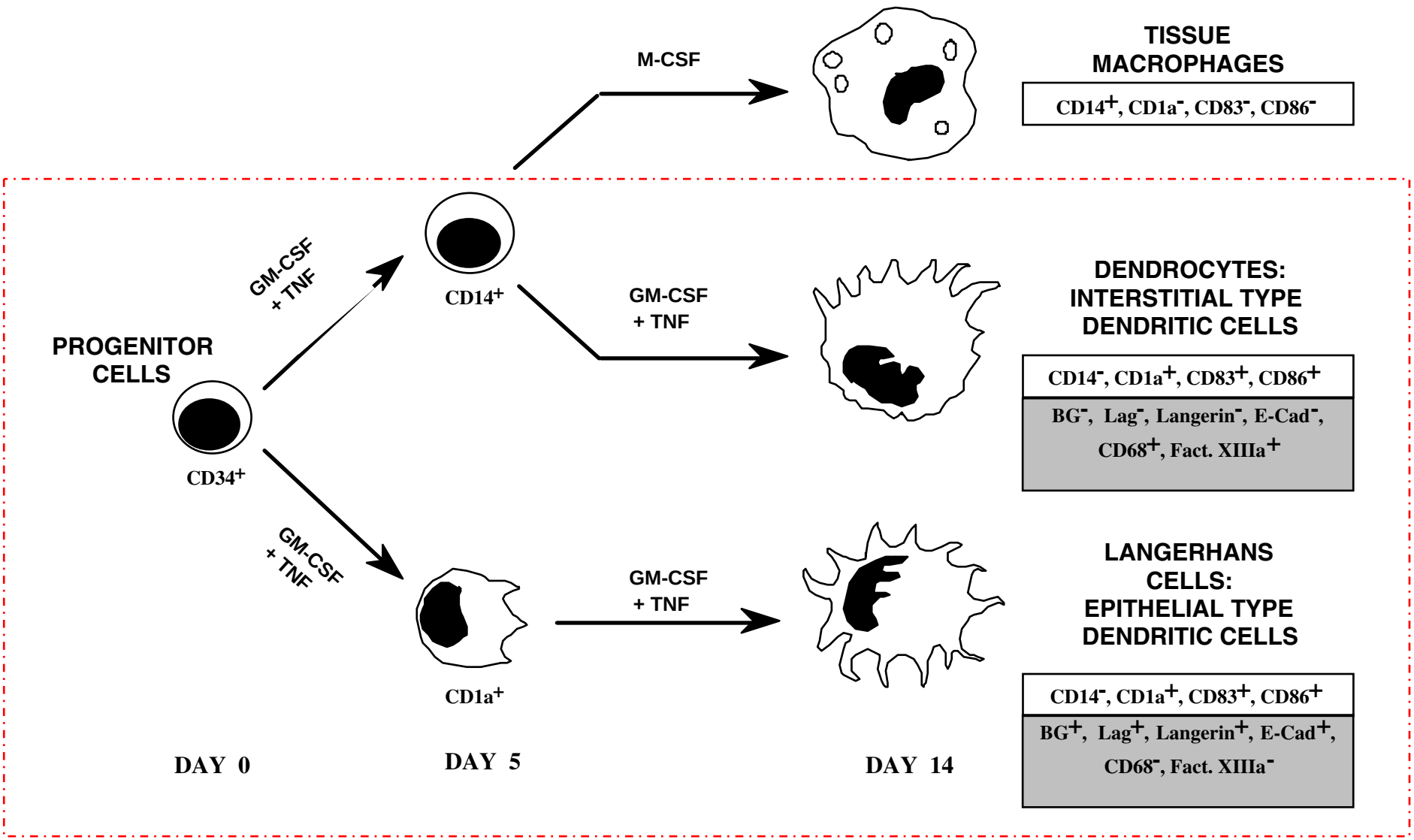
Sources of DC in Clinical Trials

- **Purified from Peripheral Blood**
- **CD34+ cell-derived (GM-CSF + TNF)**
- **Monocyte-derived (GM-CSF + IL-4)**

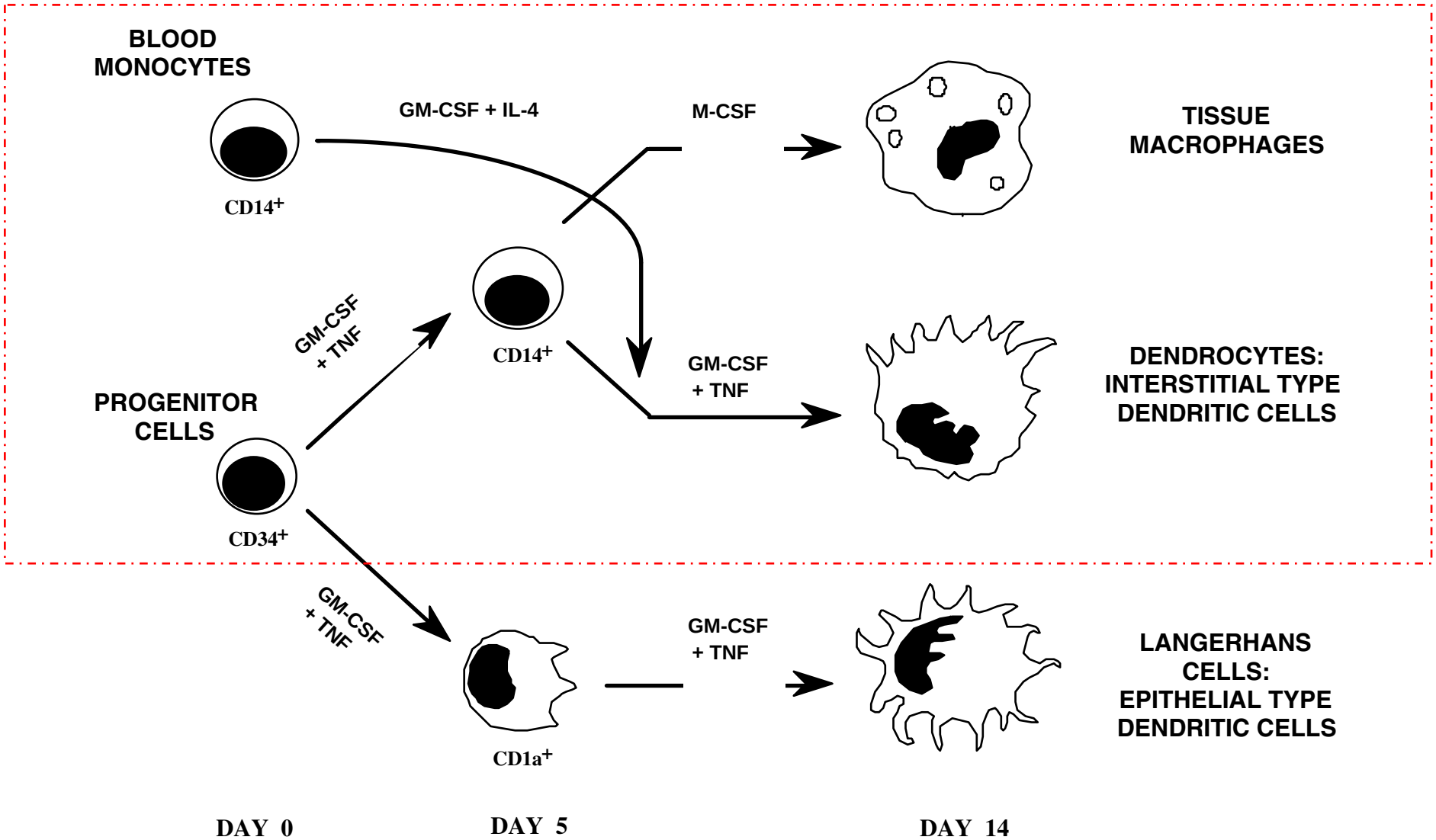




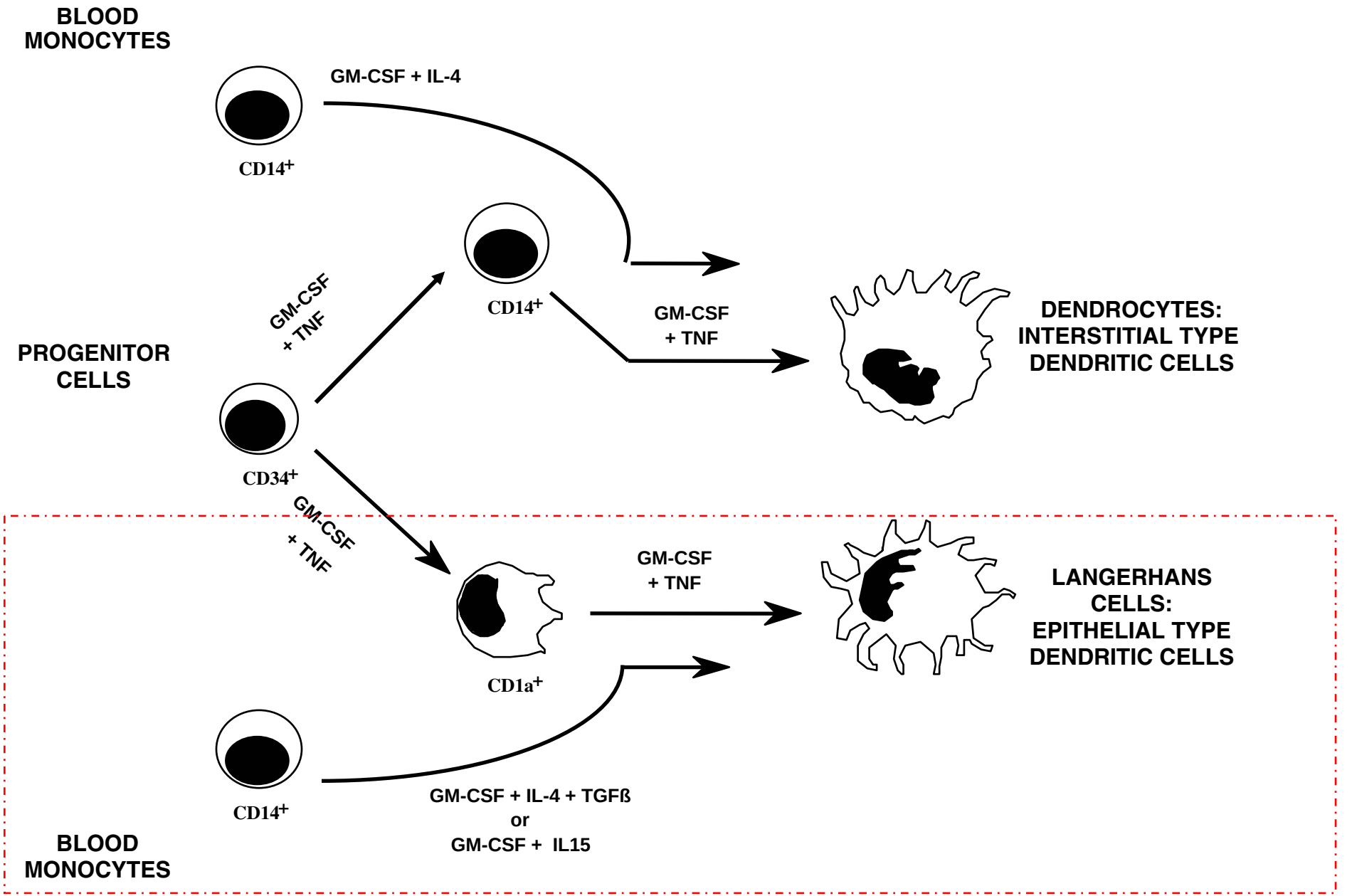
Two pathways of dendritic cell development from CD34 hematopoietic progenitors

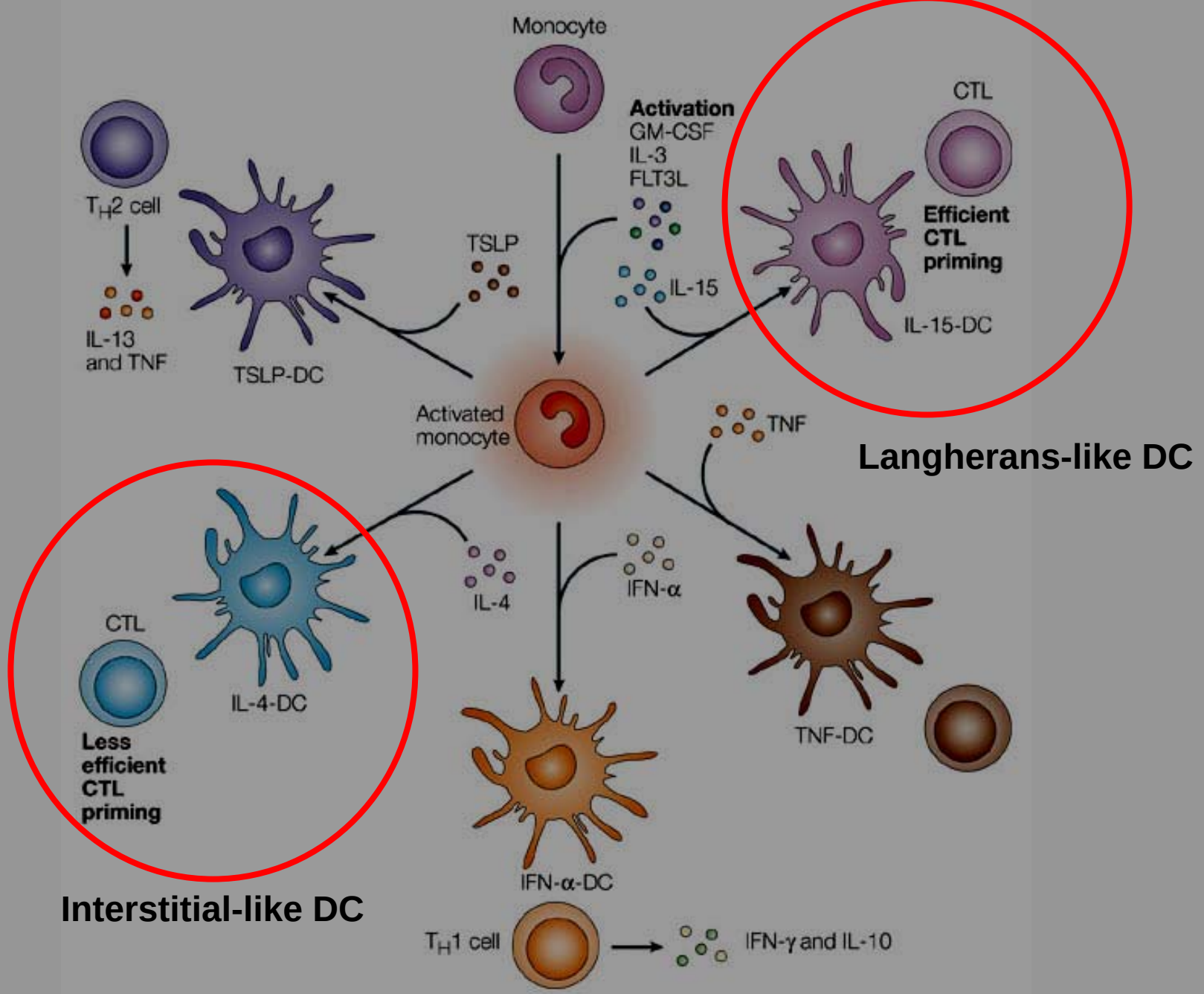


In vitro development of dendritic cells from CD34 hematopoietic progenitors or from monocytes



In vitro development of Langerhans cells



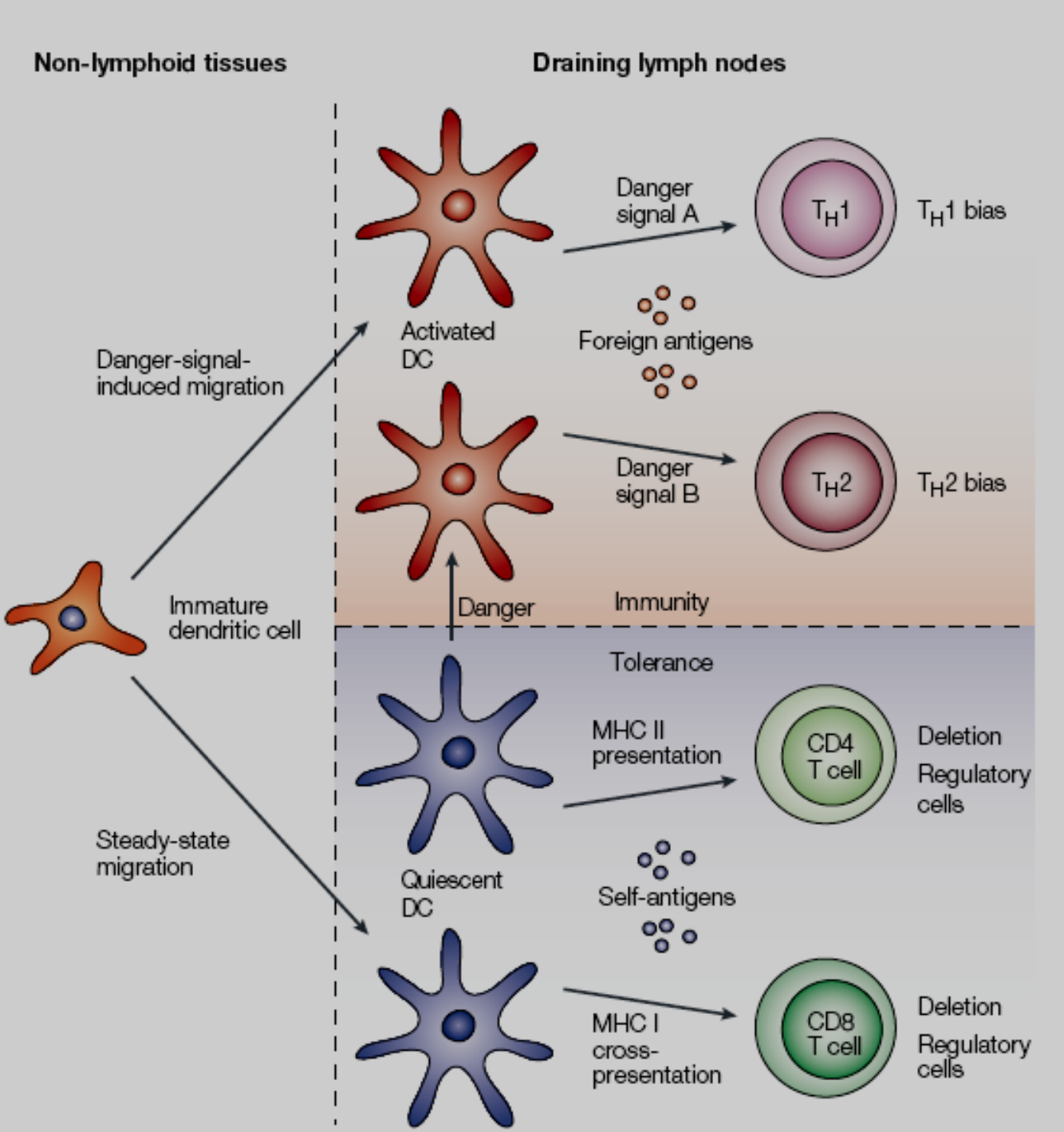


Modified from: Bancherau and Palucka, 2005

Nature Reviews | Immunology

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

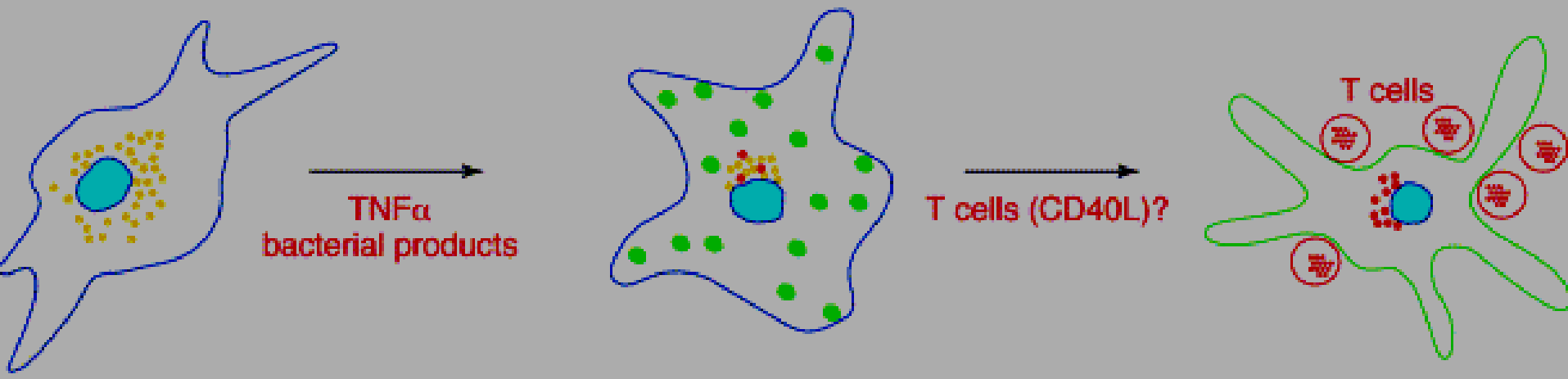
→

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

Immature cells
peripheral tissues

Intermediate cells
peripheral tissues, afferent lymph?

Mature cells
lymphoid organs



MHC class II:
- Transport to lysosomes (MIIC)
- Little surface appearance
- Rapid degradation of MHC-II

Endocytosis:
- Phagocytosis
- Macropinocytosis
- Clathrin-mediated?

MHC class II:
- Peripheral non-lysosomal structures (CIIV)
- Peptide loading
- Transport to surface

Endocytosis:
- ?

MHC class II:
- Most on plasma membrane
- Peptide loaded
- Long lived

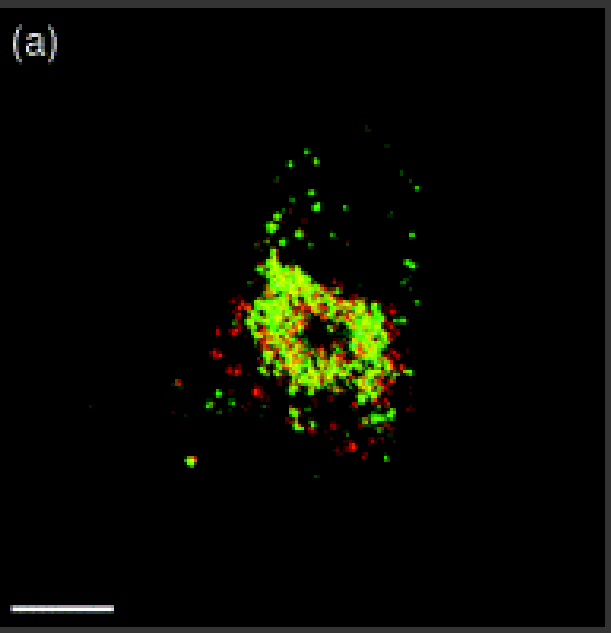
Endocytosis:
- No phagocytosis
- No macropinocytosis
- Clathrin-mediated?

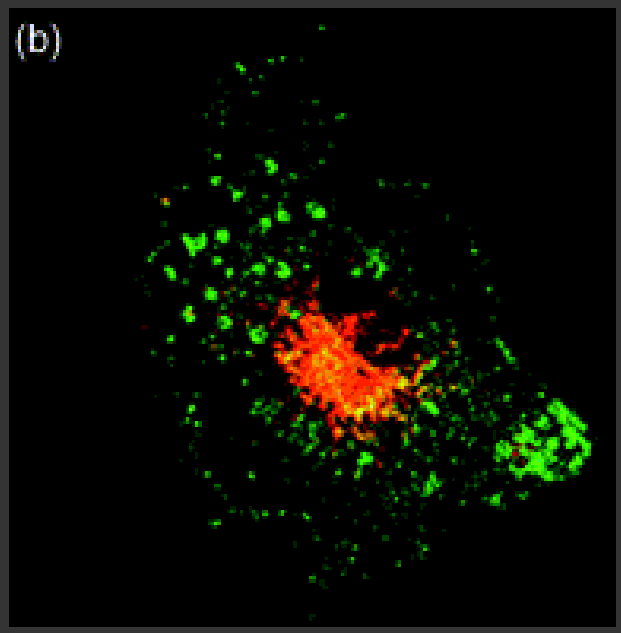
Modified from Mellman I et al, Trends in Cell Biology 1998

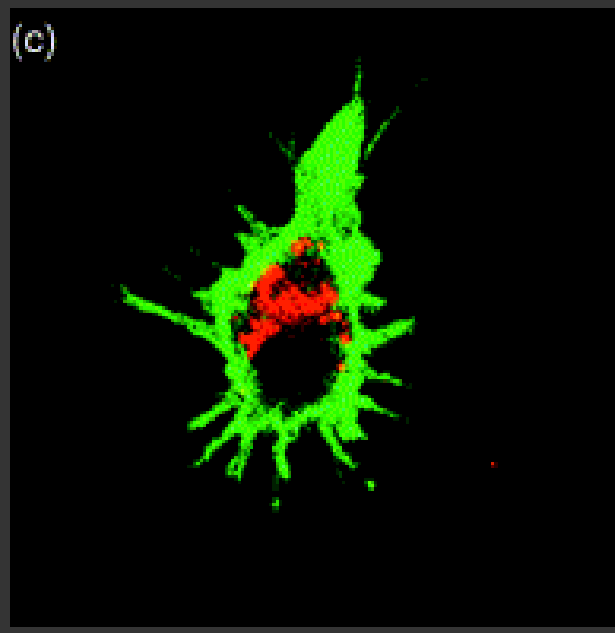
Immature DC

Intermediate DC

Mature/activated DC







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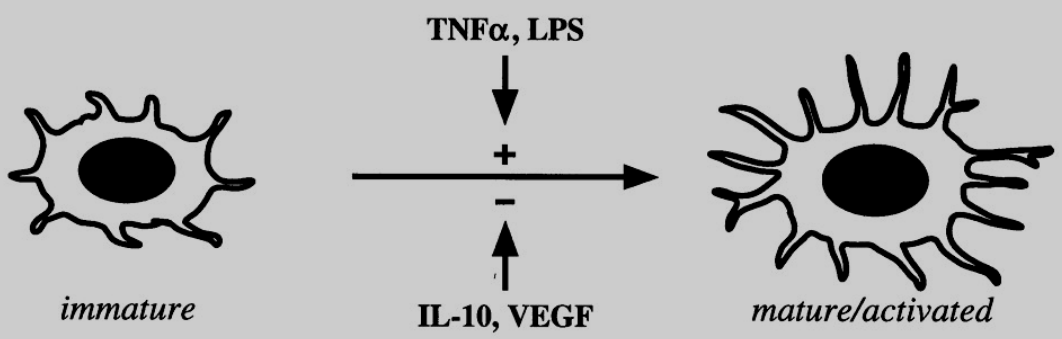


+	Antigen presentation molecules **MHC class I and II, CD1**	+++	**Signal 1**
++	Receptors for antigen uptake Mannose receptor, DEC-205, Fcγ receptors	+/–	**Antigen uptake**
++	Endocytotic activity	+/–	
+/–	Motility	++	**Migration**
+	Adhesion molecules CD11a,b,c, CD50, CD54, CD58	+++	
+	Costimulatory molecules CD80/B7.1, CD86/B7.2, CD40	+++	**Signal 2**
–	Secretions IL-12, T cell attractant chemokine	+++	**Signal 3**
–	Leukocyte differentiation antigens CD3, CD8, CD19, CD20, CD56	–	
–	CD83	++	

Timmerman JM, Levy R.

Dendritic cell vaccines for cancer immunotherapy.

Annu Rev Med. 1999;50:507

Maturation/Activation of DC in Clinical Trials

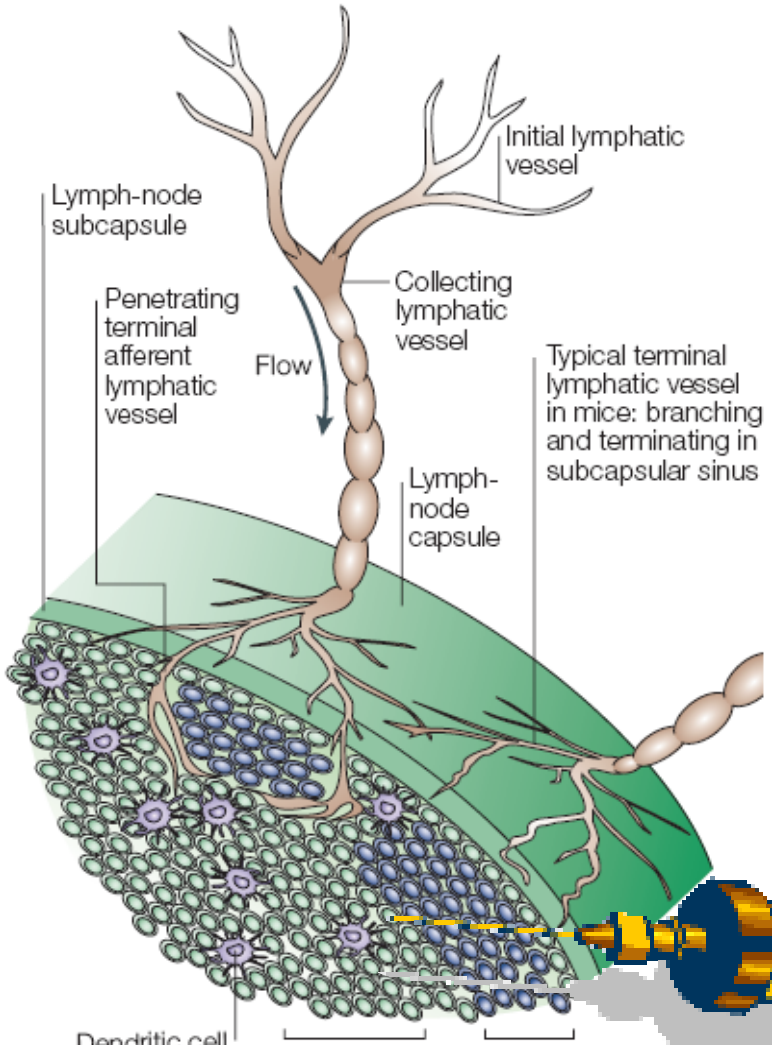
- **Monocyte-conditioned medium**
- **TNF-α, IL-1β, IL-6, PGE2**
- **Trance/RANKL, CD40L**

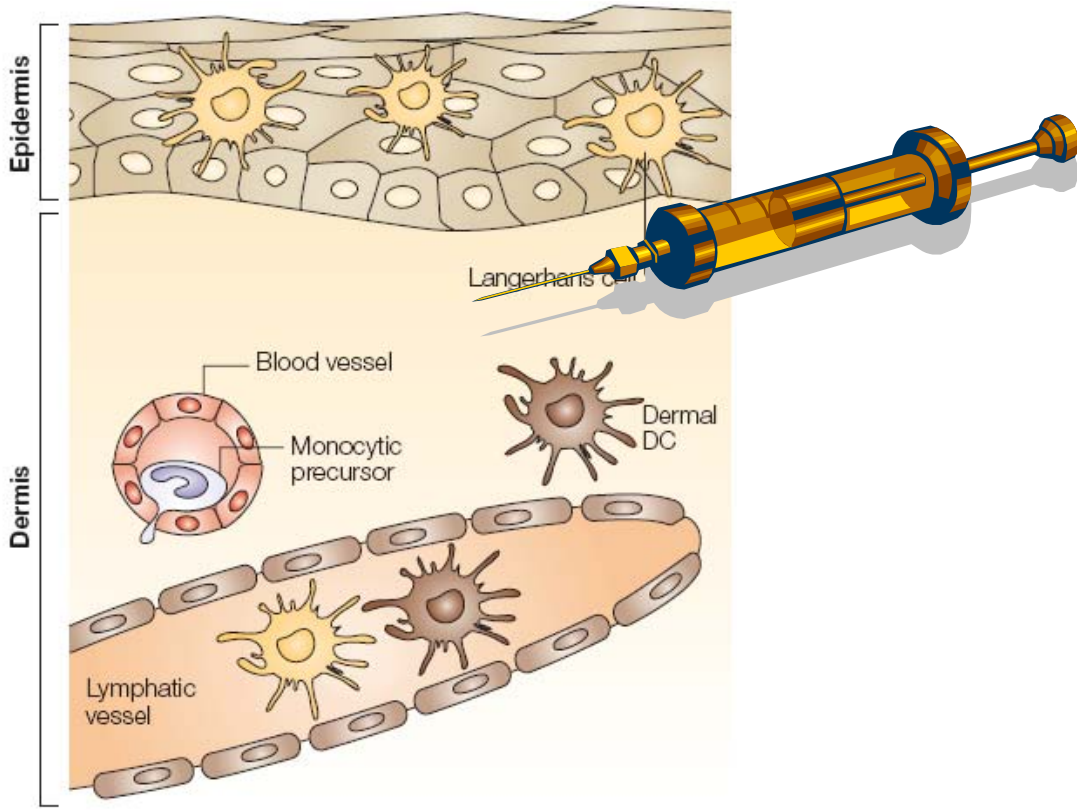
Variables in DC adoptive therapy

- 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

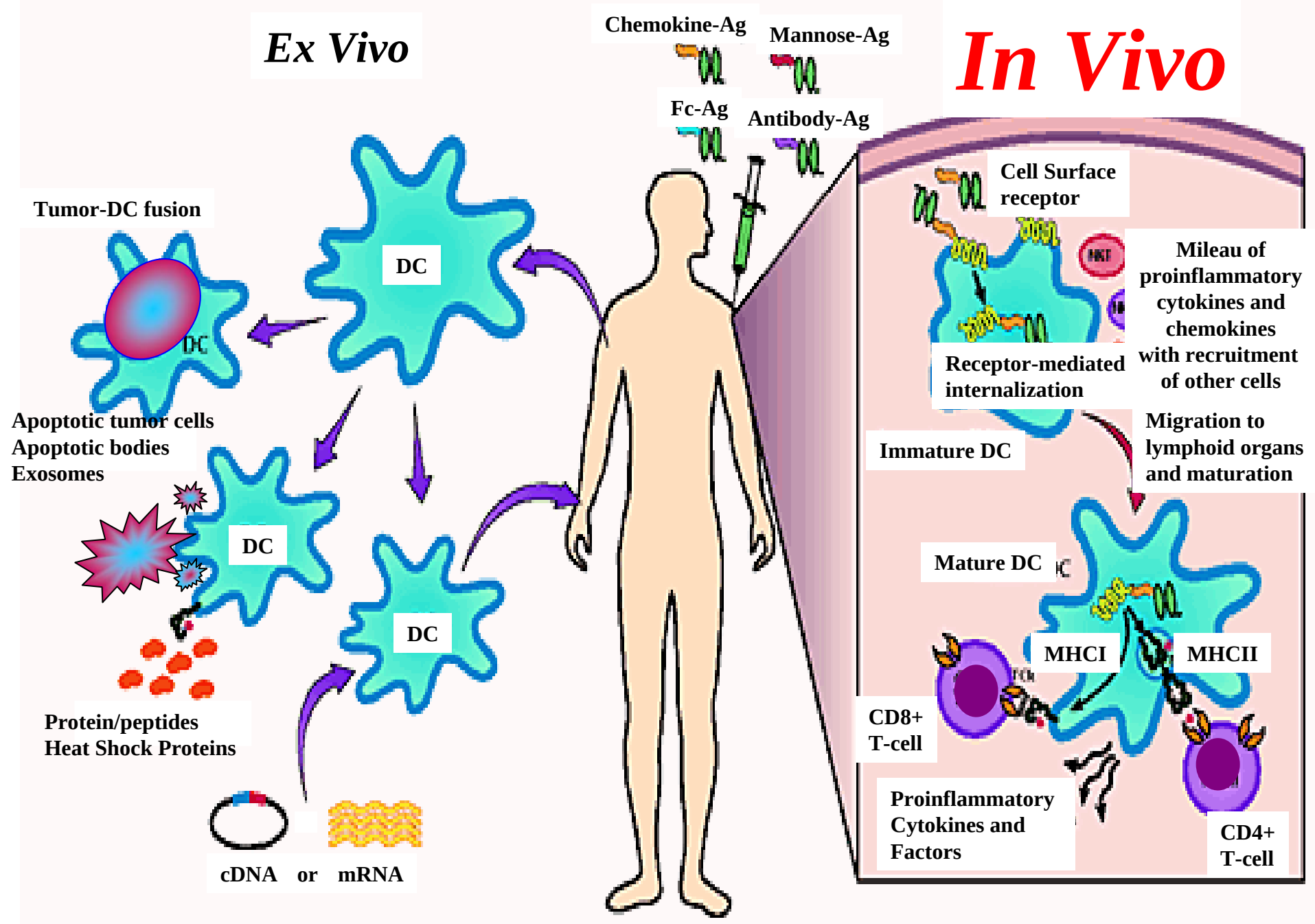
DC adoptive therapy / vaccination in cancer patients is safe, often induces an immune response against tumor-associated antigens, and in a proportion of cases induces a lasting partial or rarely complete remission that has been correlated with the extent of the immune response generated.

Clinical outcome of cancer vaccines in patients with melanoma

Vaccine	Total patients	Responding patients	Response rate (%)
Peptide vaccines	410	11	2.7
Viral vectors	160	3	1.9
Tumour cells	43	2	4.6
Dendritic cells	116	11	9.5

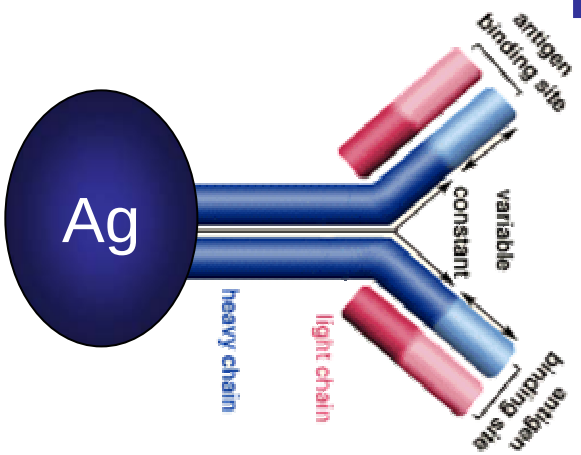
Data taken from **(Rosenberg et al, Nature Medicine, 2004)**

From: Bancherau and Palucka, Nature Review Immunology, 2005



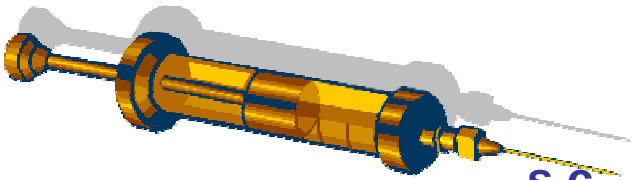
Biragyn & Kwak: Nature Med. 6:966 (2000) (modified)

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

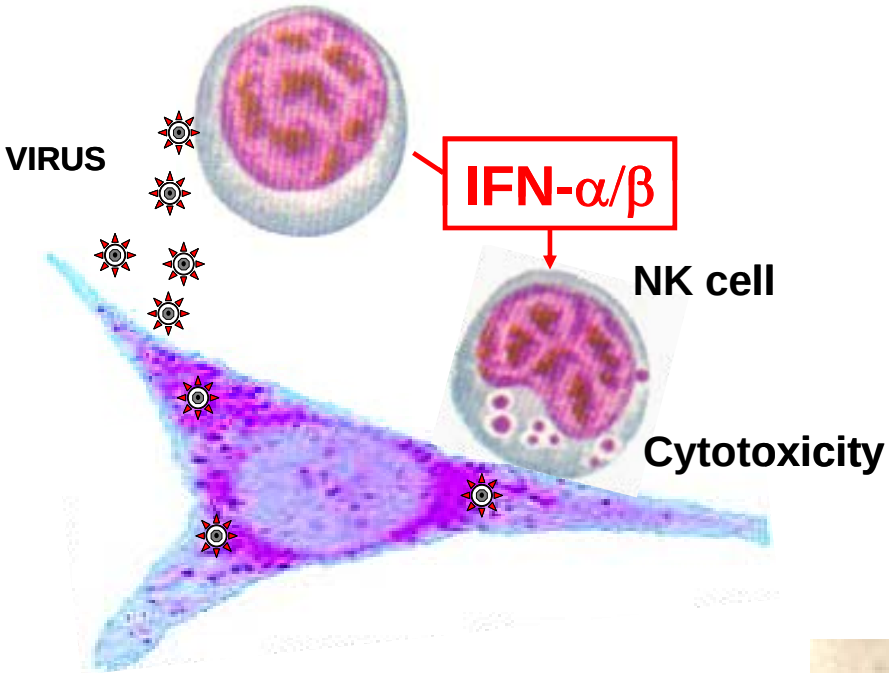
Mature DC → IMMUNITY,
TUMOR REJECTION

Bonifaz L, Bonnyay D, Mahnke K, Rivera M, Nussenzweig MC, Steinman RM. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. J Exp Med. 2002 196:1627-38.

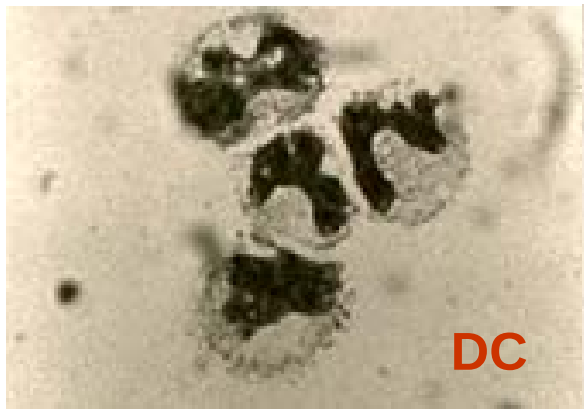
Mahnke K, Qian Y, Fondel S, Brueck J, Becker C, Enk AH. Targeting of antigens to activated dendritic cells in vivo cures metastatic melanoma in mice. Cancer Res. 2005 65:7007-12.

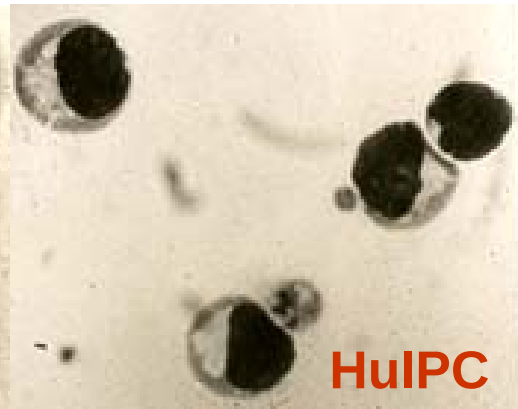
Plasmacytoid precursor dendritic cells or type I Interferon producing cells.

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

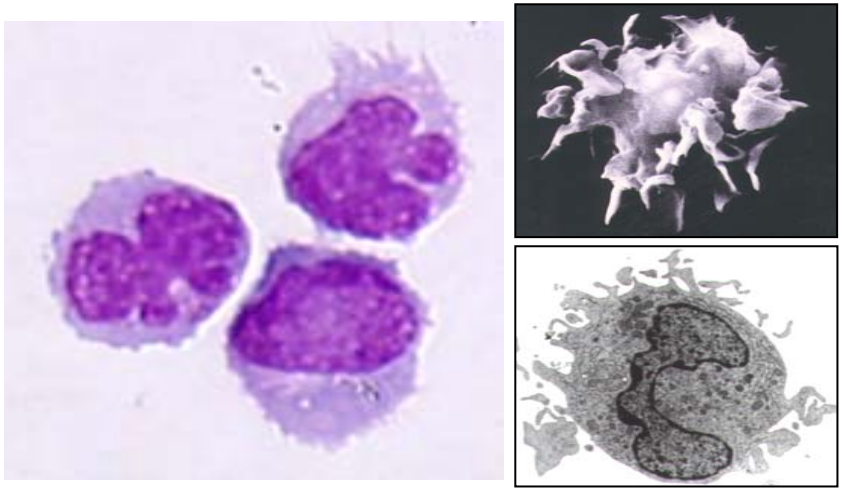


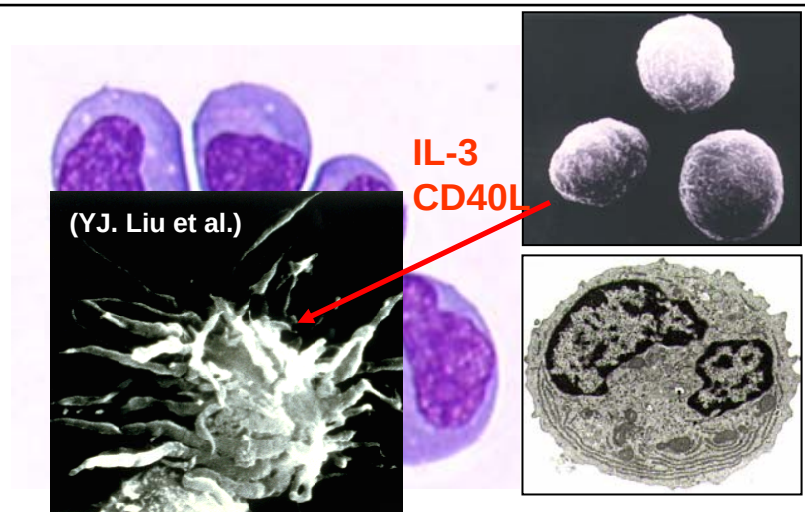
- IPC are the only cell type in human blood able to produce type IFN in response to viruses
- IPC represent 1/500 to 1/200 of PBMC
- IPC are MHC class II positive but distinct in functions and morphology from Dendritic Cells
- IPC are very poor APC but are required for NK cell-mediated killing of virus-infected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

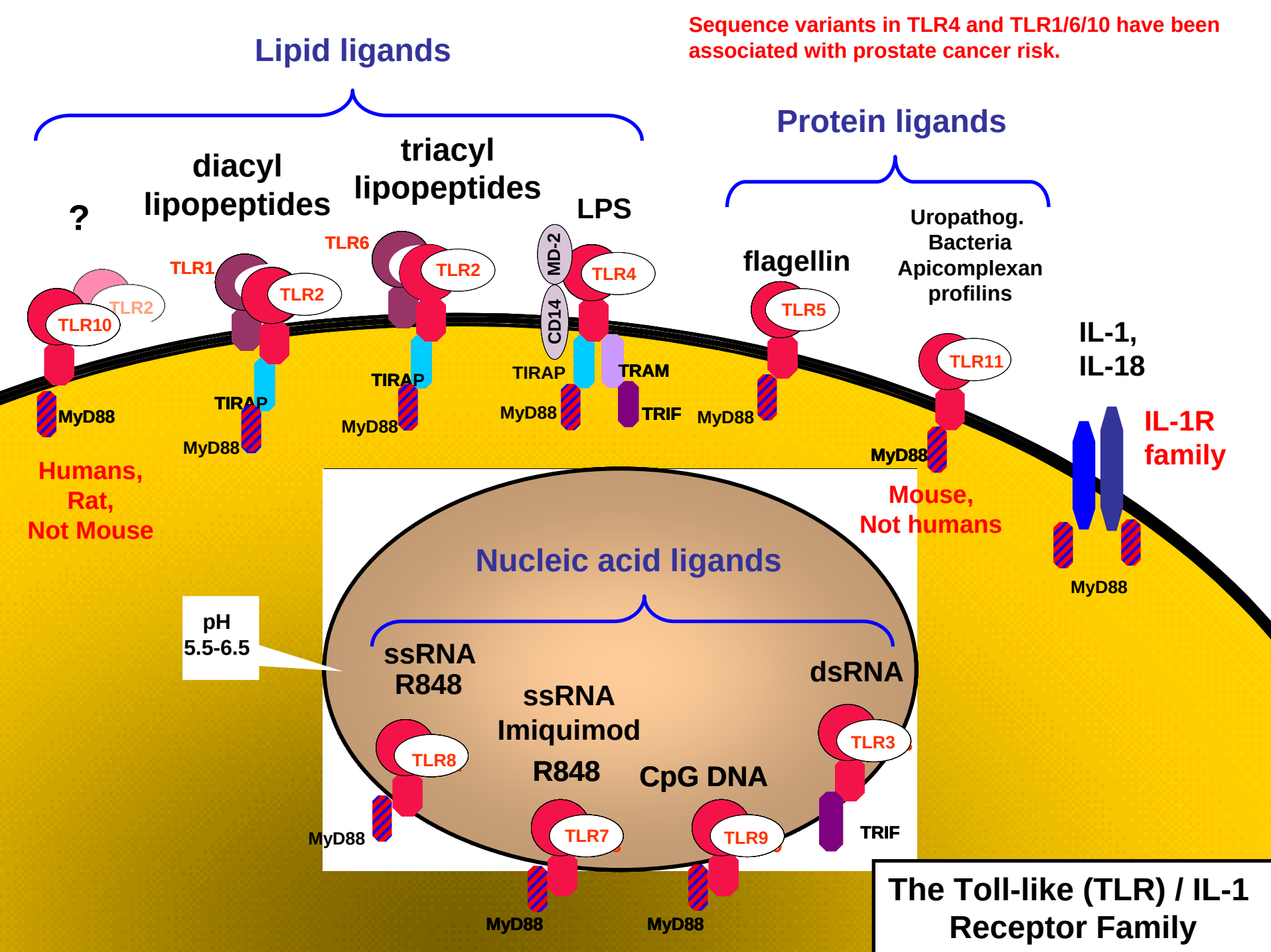
(Liu, Briere, Colonna, 1999)

Type I IFN producing cells (70-80s)

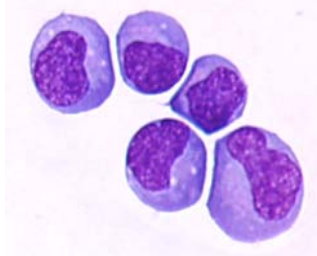
High titer of IFN-α in response to viruses and certain CpG

Low IL-12 secretion

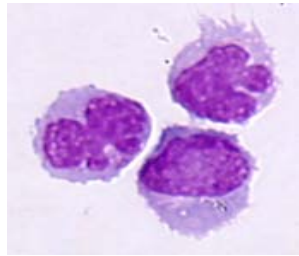
TLR 2± 3+ 4± 7± 8+ 9- 10- TLR 2- 3-4- 7+ 8- 9+ (10+)


Sequence variants in TLR4 and TLR1/6/10 have been associated with prostate cancer risk.
Lipid ligands
Protein ligands
?
diacyl lipopeptides
triacyl lipopeptides
LPS
flagellin
Uropathog. Bacteria Apicomplexan profilins
IL-1, IL-18
IL-1R family
TLR2
TLR10
TLR1
TLR2
TLR6
TLR2
MD-2
CD14
TLR4
TLR5
TLR11
MyD88
TIRAP
MyD88
TIRAP
MyD88
TIRAP
TRAM
MyD88
TRIF
MyD88
MyD88
Humans, Rat, Not Mouse
Mouse, Not humans
Nucleic acid ligands
pH 5.5-6.5
ssRNA R848
ssRNA Imiquimod R848
CpG DNA
dsRNA
TLR8
TLR7
TLR9
TLR3
TRIF
MyD88
MyD88
MyD88
The Toll-like (TLR) / IL-1 Receptor Family

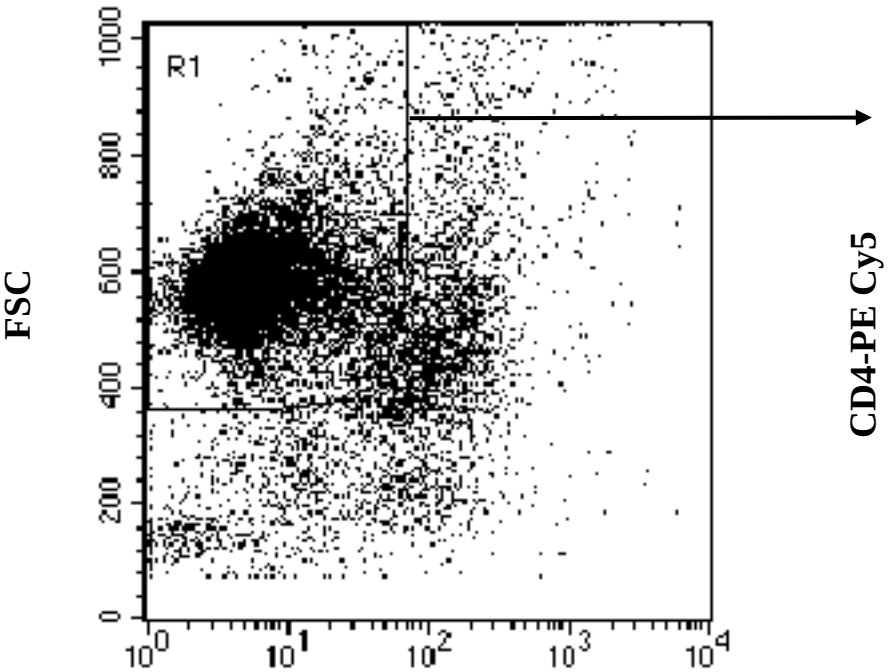
Human dendritic cell subsets

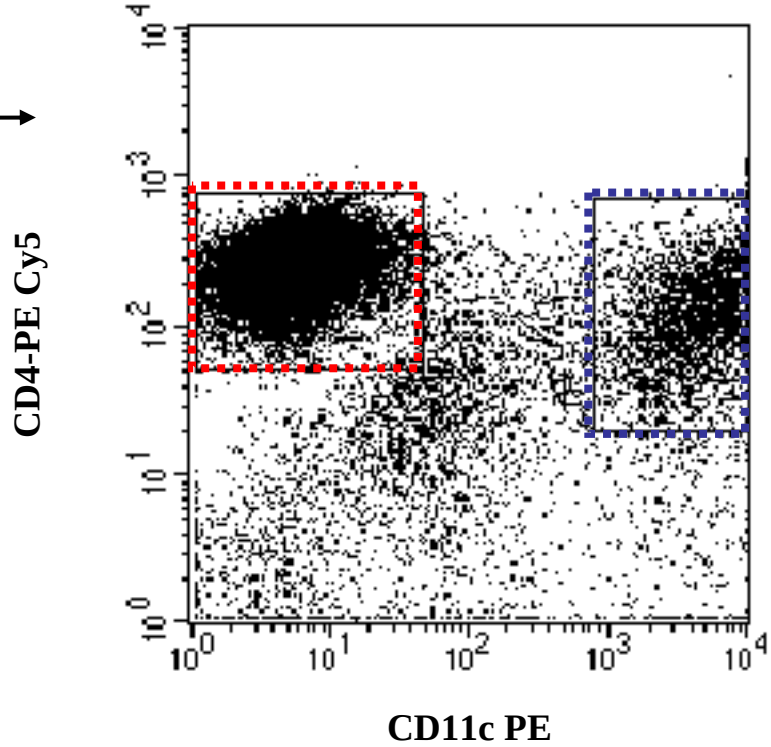


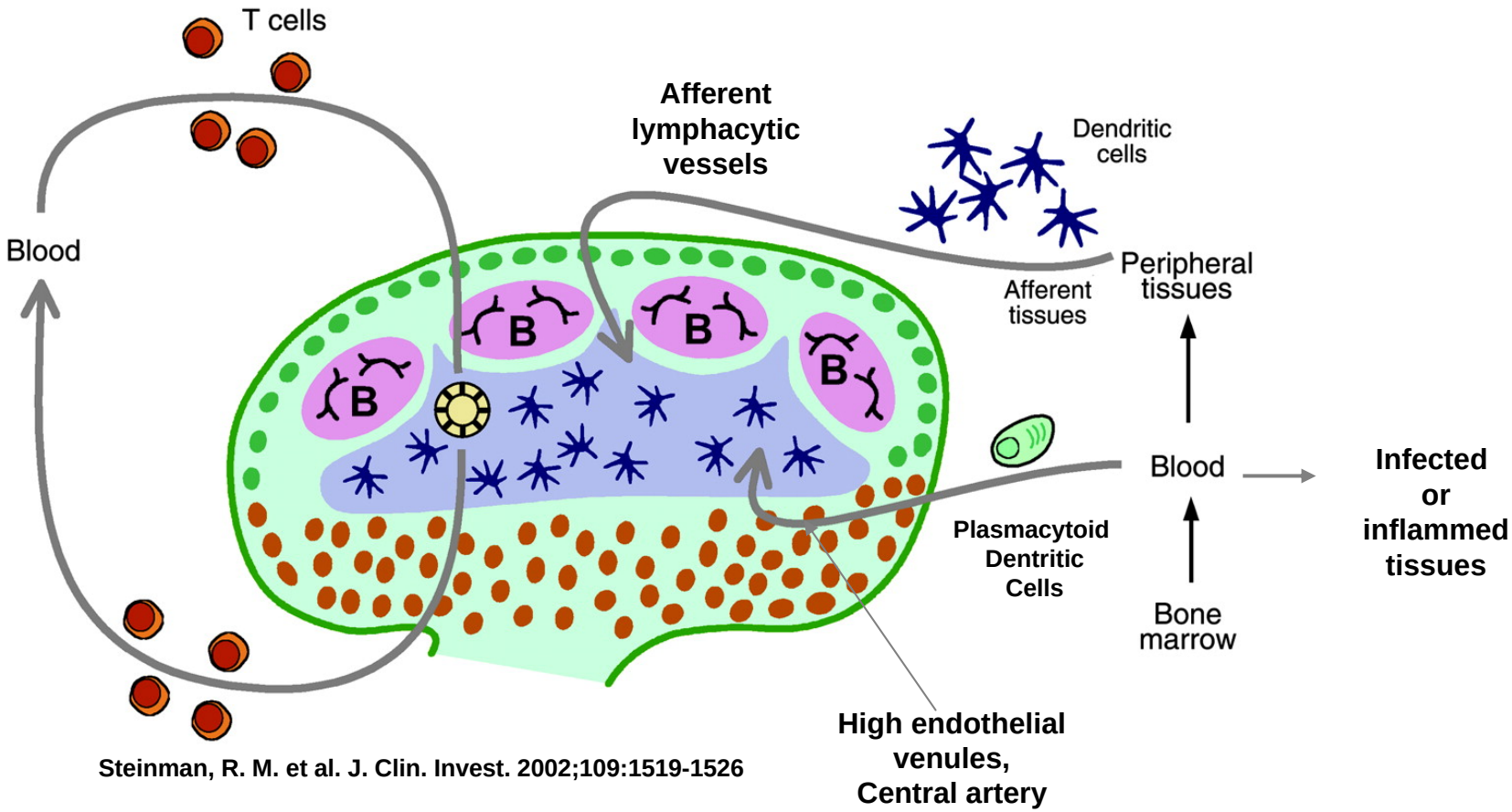
Plasmacytoid DC
ILT3⁺ ILT1⁻
BDCA-2



Myeloid DC
ILT3⁺ ILT1⁺
BDCA-1=CD1c







Steinman, R. M. et al. J. Clin. Invest. 2002;109:1519-1526

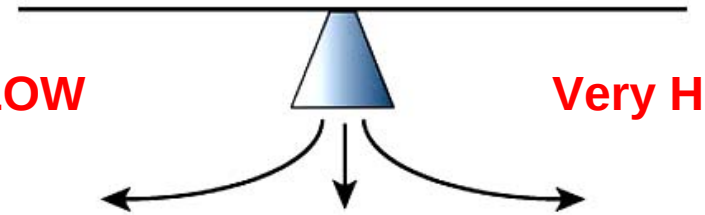
Plasmacytoid Dendritic Cells

- **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.**

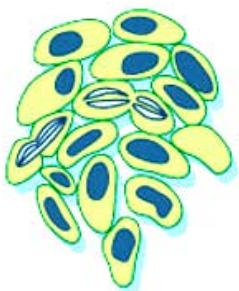
Physical, mechanical cause
Auto-immunity
Infection
Endogenous factors

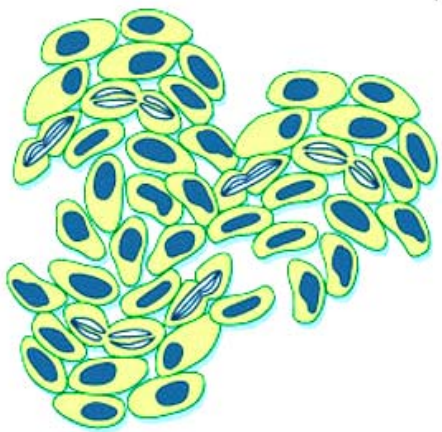
TUMOR

INFLAMMATION: Very LOW — Very HIGH



Few chemokines (e.g. ELR(+)CXC)	Abundant pro-inflammatory chemokines (e.g. ELR(+)CXC)	Altered balance of pro- and anti-inflammatory chemokines (e.g. ELR(–)CXC)
Limited inflammation	Inflammation	Excessive inflammation
Restricted vascularization	Neovascularization	Angiostasis
Restricted tumour growth	Rapid tumour growth	Tumour regression





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-angiogenis effects.

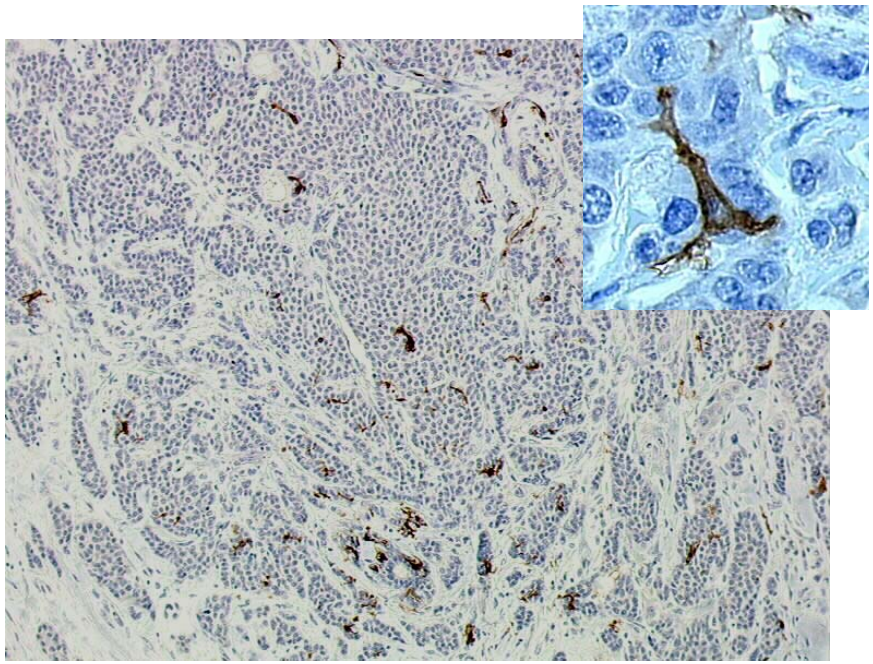
TLR-ligands have anti-tumor activity that is enhanced by antagonism of IL-10.

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.

Modified from Coussens L.M. and Werb Z.
Nature 2002, 420:860-867

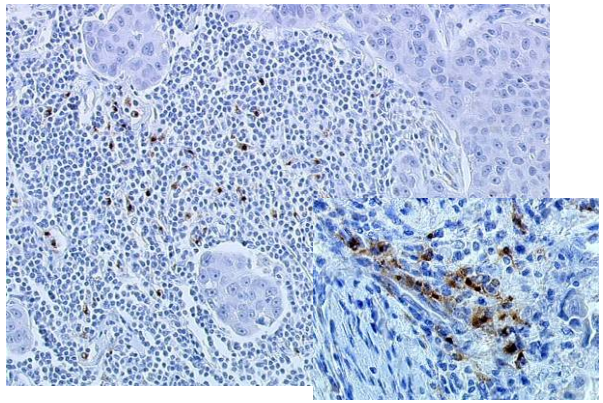
CD1a$^+$ DC in breast cancer are in direct contact with tumor cells



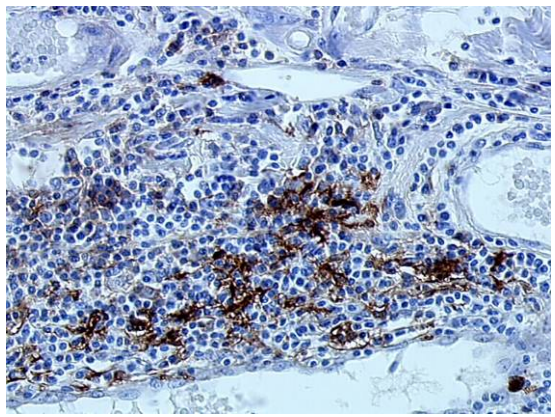
(~30% positive tumors)

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

DC-Lamp +



CD40 +



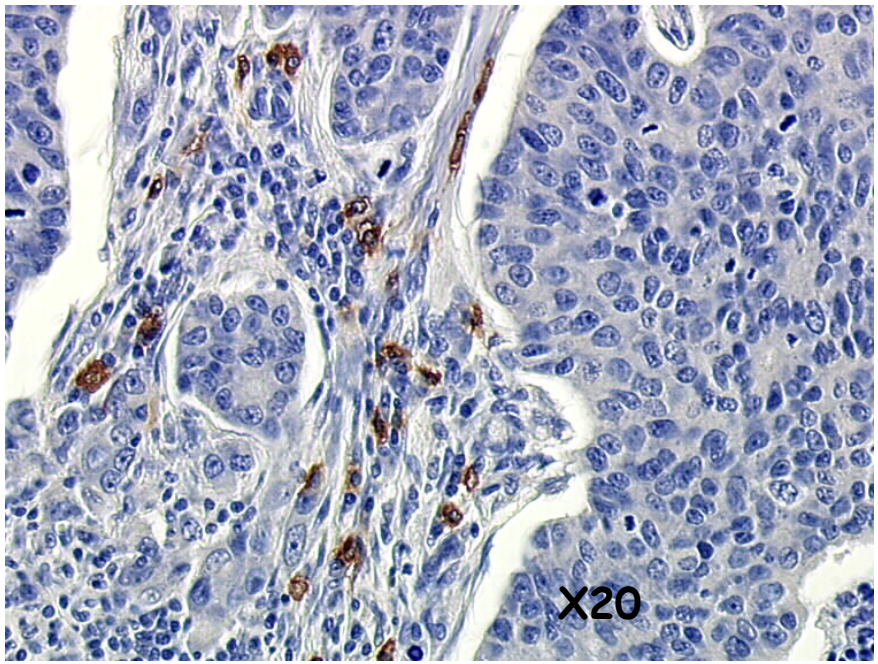
(~50% positive tumors)

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

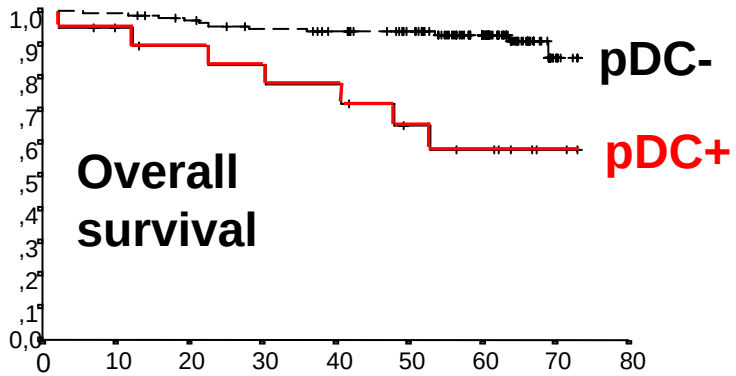
Treilleux I et al. Dendritic cell infiltration and prognosis of early stage breast cancer.2004. Clin Cancer Res. 10:7466-74.

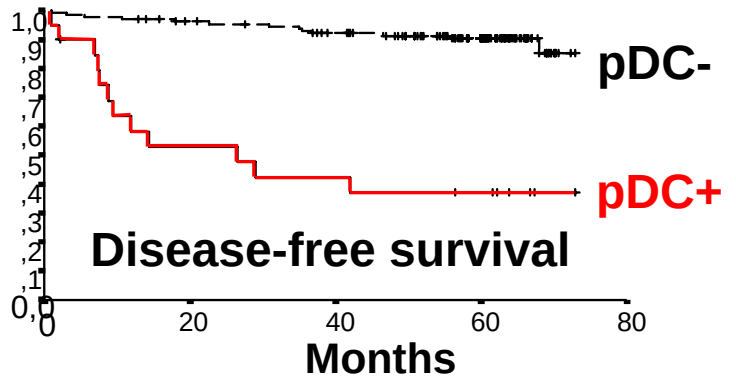
Tumor-infiltrating Dendritic Cells: Friends or Foes?

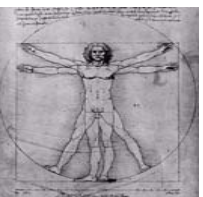
CD123^{+}DC (and BDCA-2+) plasmacytoid DC) infiltrate human breast tumors in 13% of the patients



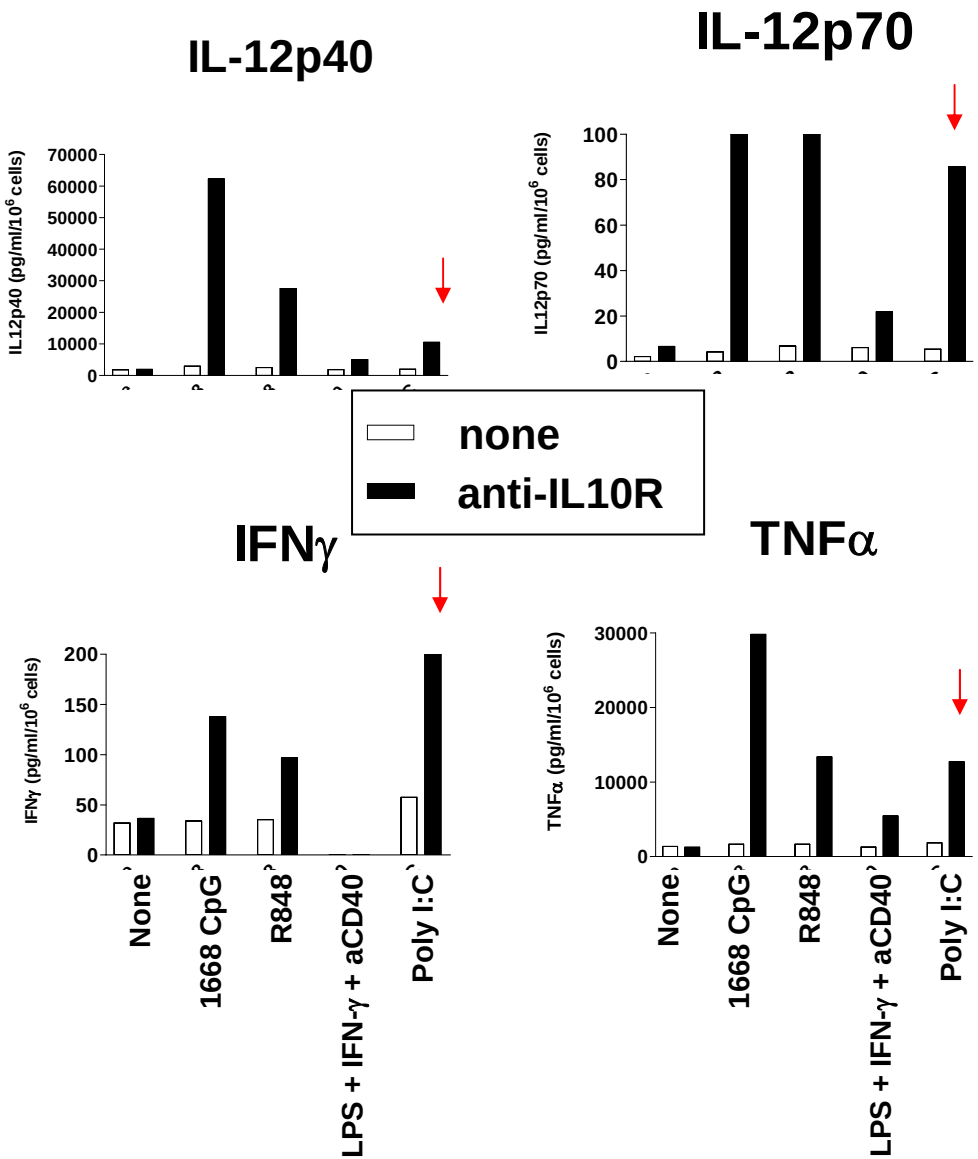
The presence of pDC in the primary tumor strongly correlates with poor prognosis



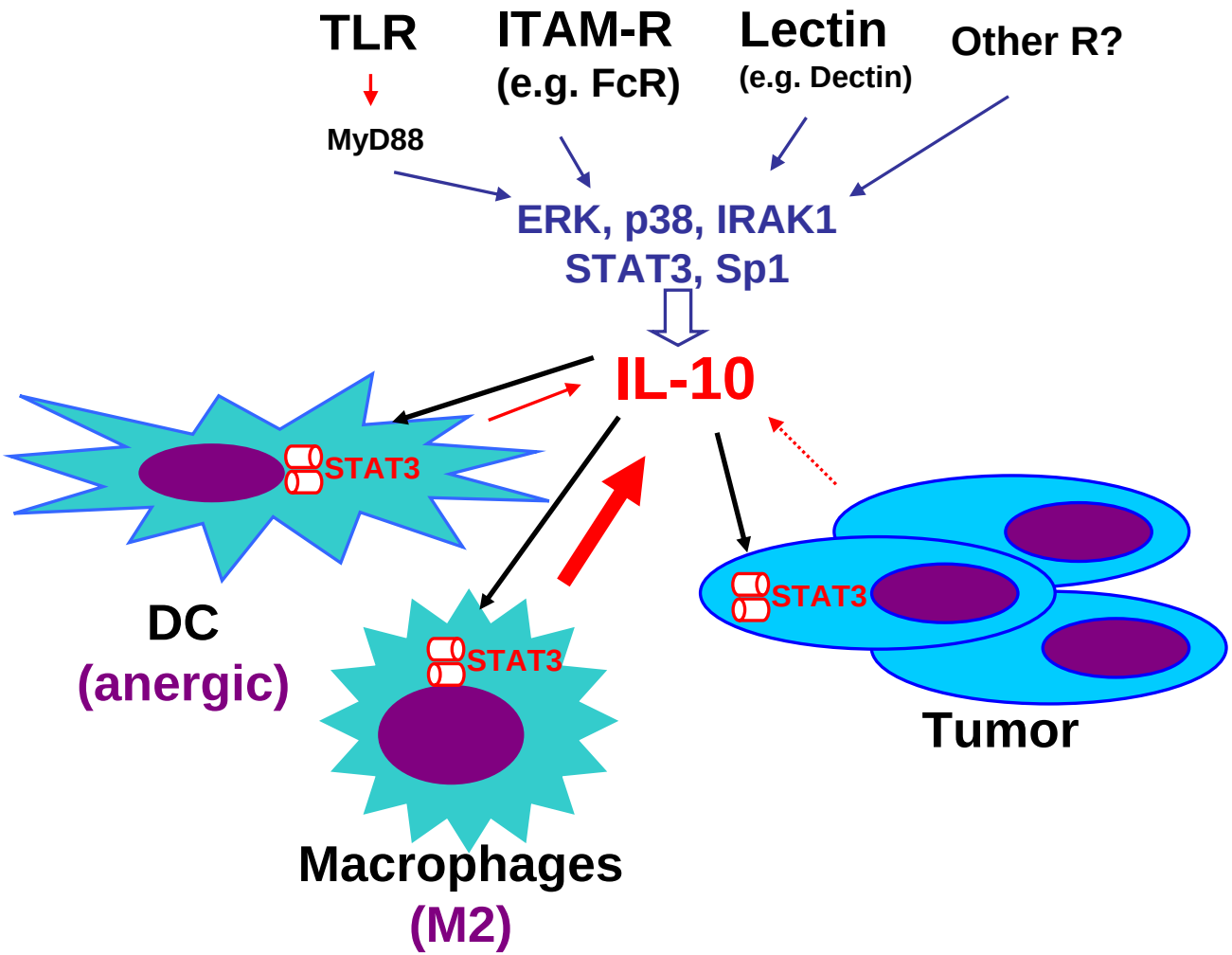




Mouse TIDC are anergic but respond to select TLR ligands when IL-10 is inhibited



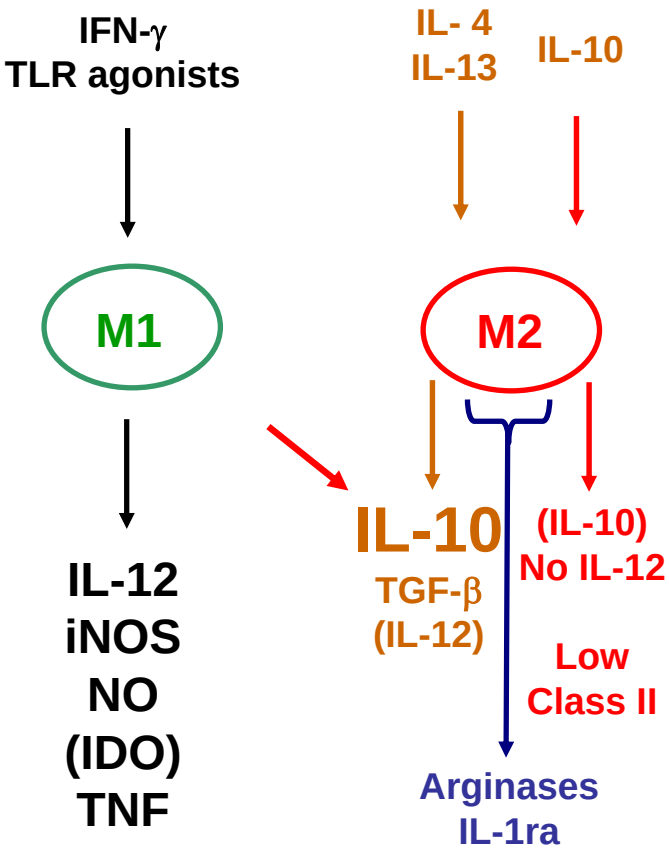
Interleukin-10 plays an important role in the immunosuppressive tumor environment.

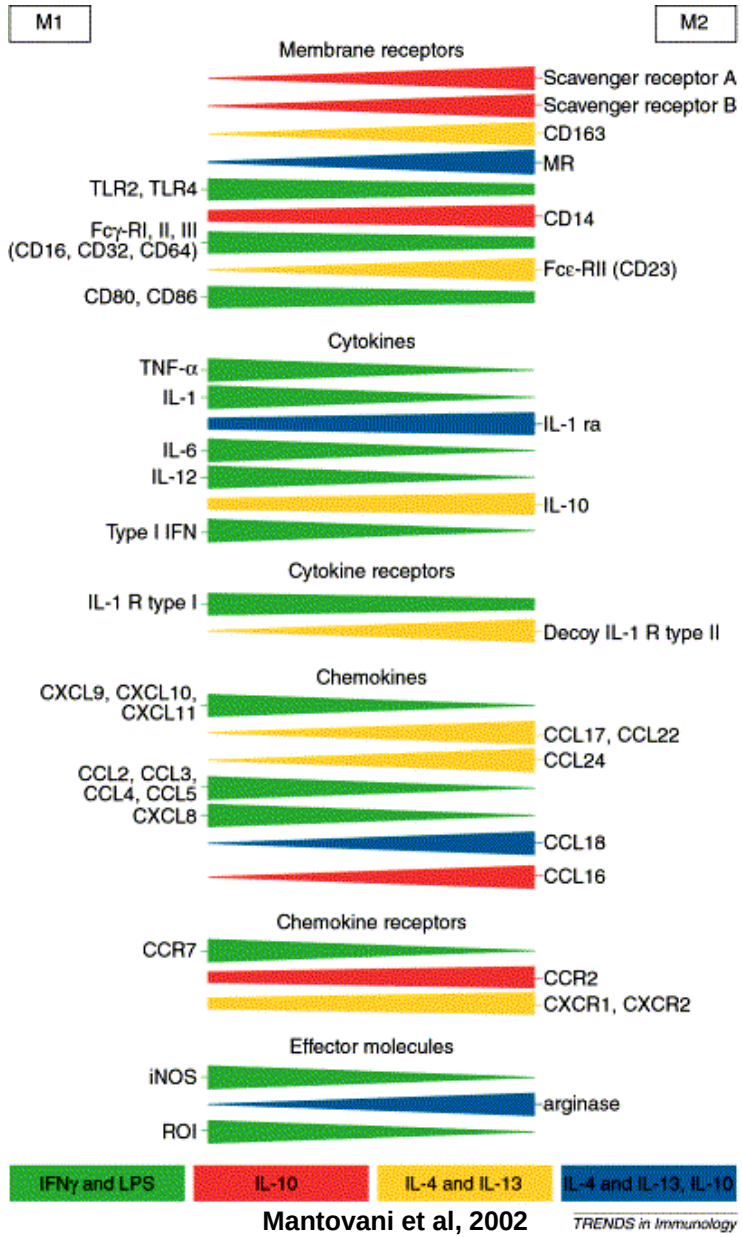


Alternative Macrophage Activation

Tumor Associated Macrophages (TAM)

[Tumor Infiltrating Dendritic Cells (TIDC)]

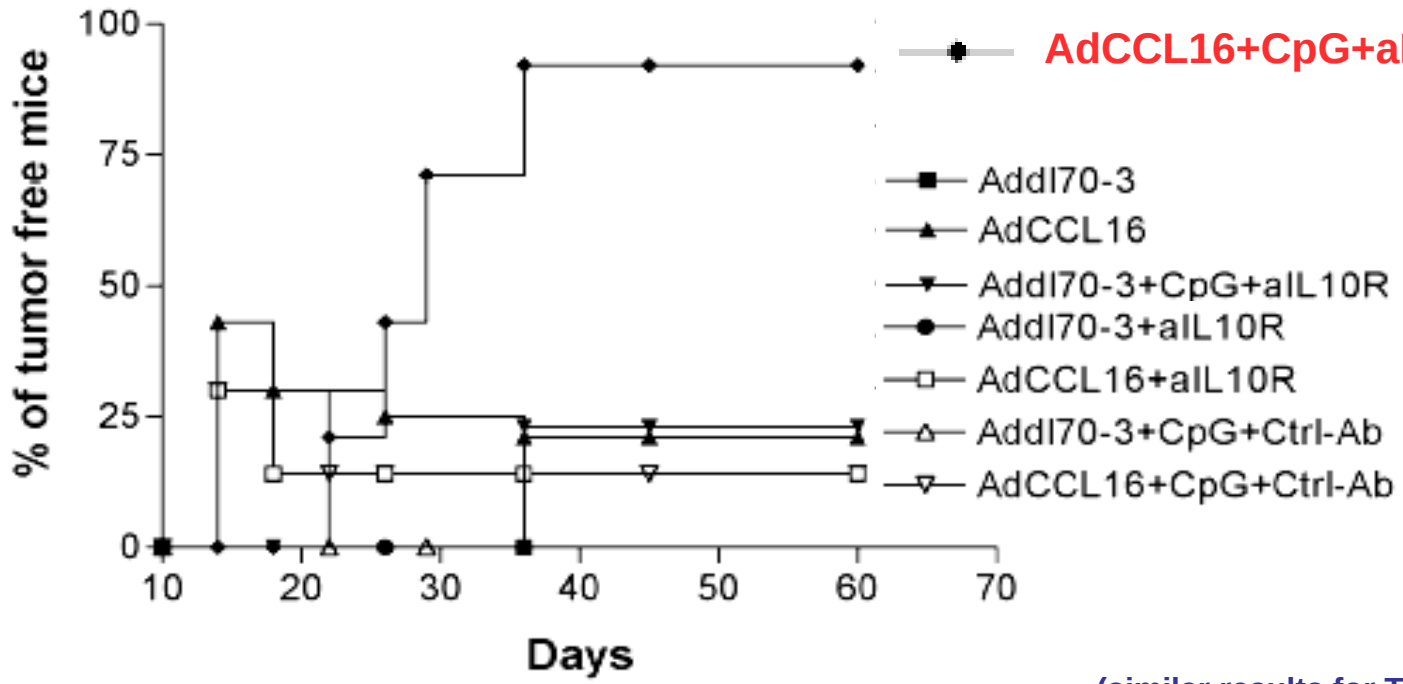




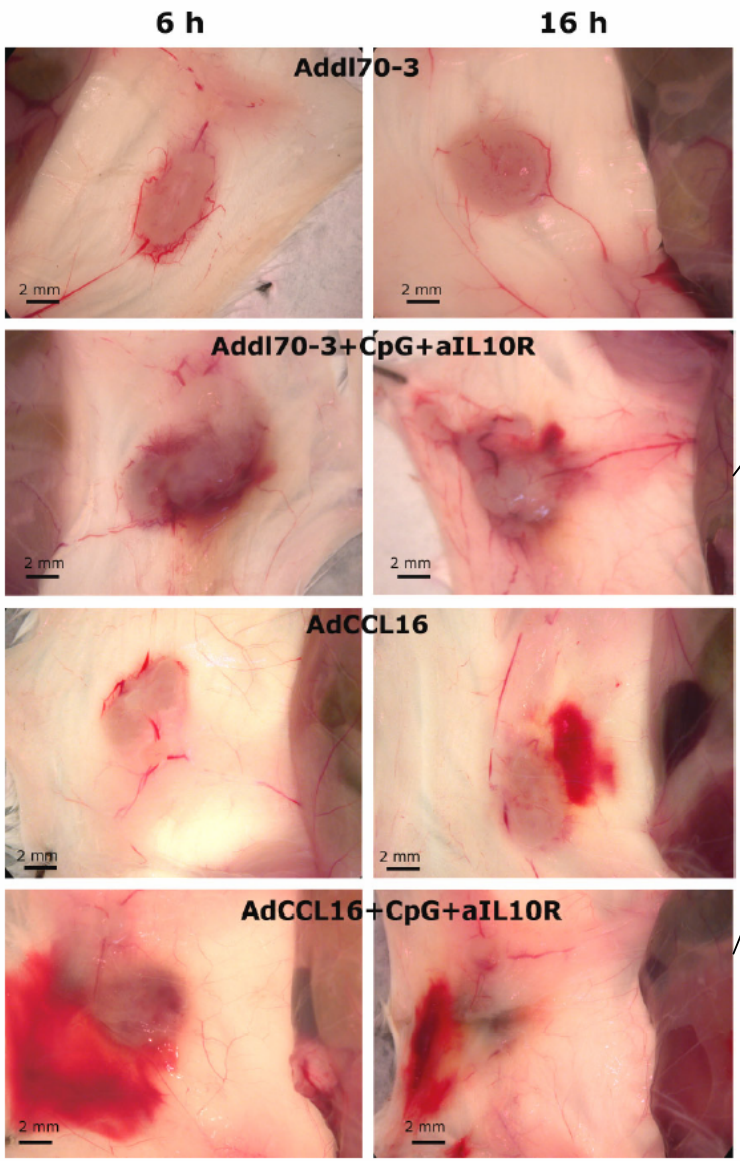
Mantovani et al, 2002

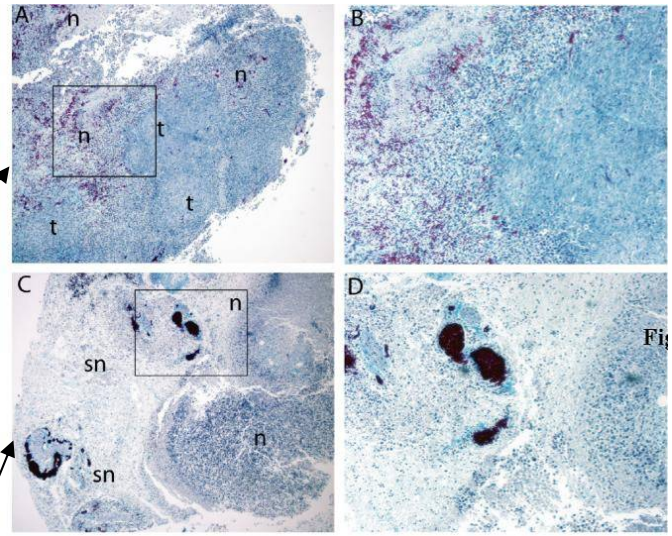
Is it possible to induce an immune response to the tumor own antigens by removing immuno-suppression and providing an inflammatory stimulus?

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

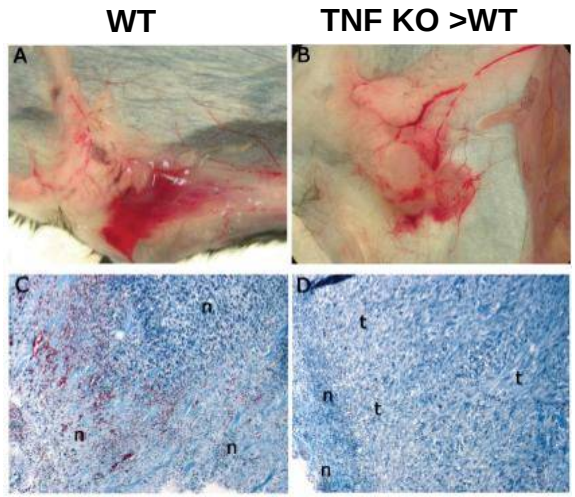


(similar results for TSA, 4T1,MC38 and for inhibition of spontaneous metastases of 4T1)



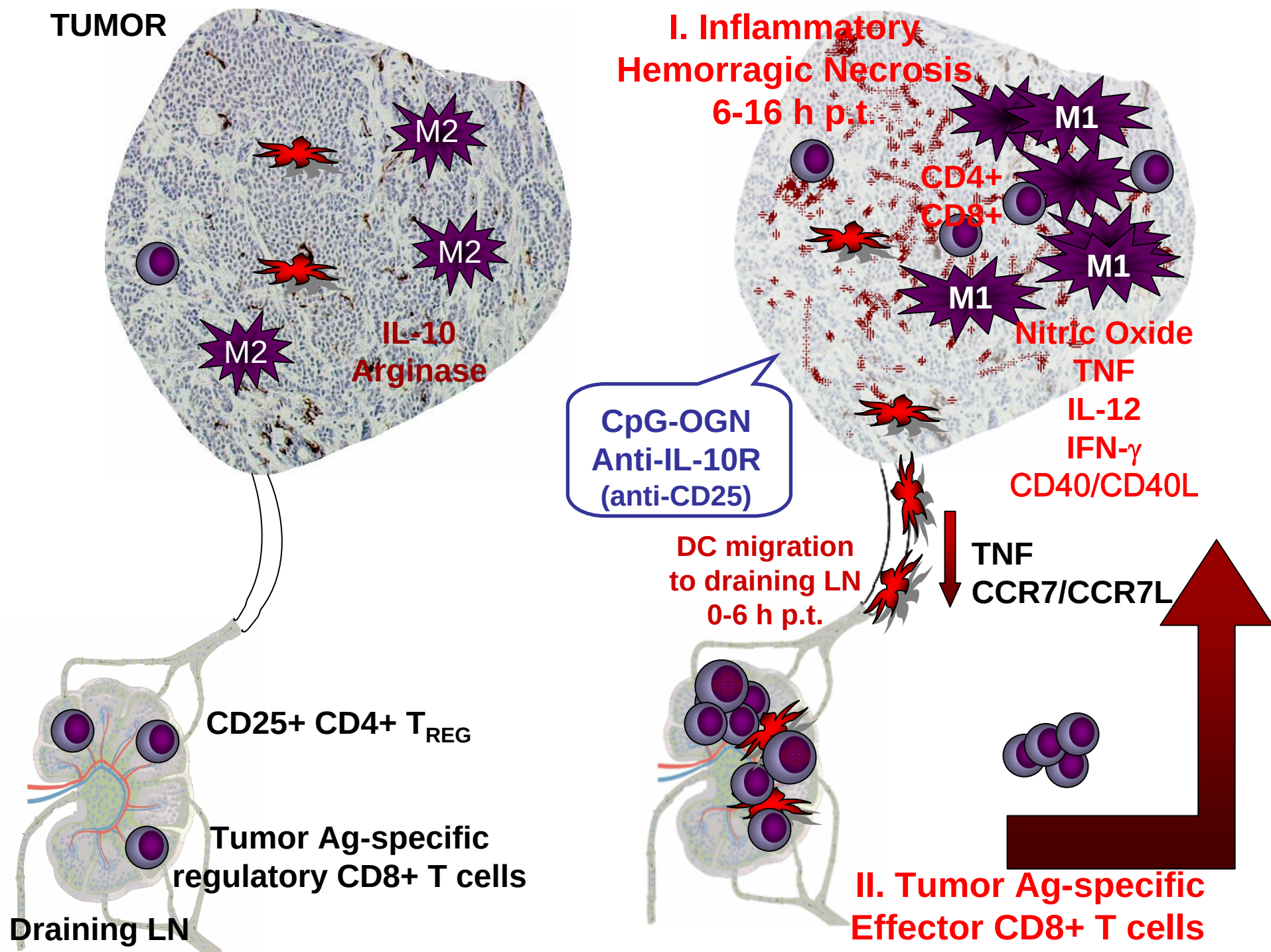


TSA



MCA38
CCL16/CpG/aIL10R
16-h p.t.

CCL16 + CpG + anti-IL-10R treatment induces a rapid hemorrhagic tumor necrosis that is dependent on TNF, IL-12, and CD40.


TUMOR
M2
M2
M2
IL-10
Arginase
I. Inflammatory
Hemorragic Necrosis
6-16 h p.t.
M1
M1
M1
CD4+
CD8+
Nitric Oxide
TNF
IL-12
IFN-γ
CD40/CD40L
CpG-OGN
Anti-IL-10R
(anti-CD25)
DC migration
to draining LN
0-6 h p.t.
TNF
CCR7/CCR7L
CD25+ CD4+ T$_{REG}$
Tumor Ag-specific
regulatory CD8+ T cells
Draining LN
II. Tumor Ag-specific
Effector CD8+ T cells

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Christophe Dercamp
Christophe Caux
Francine Brière
Serge Lebecque

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Mario Colombo
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