Dendritic Cells: Basic Biology and Therapeutic Use

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Presenter Disclosure Information

Lisa H. Butterfield, Ph.D.

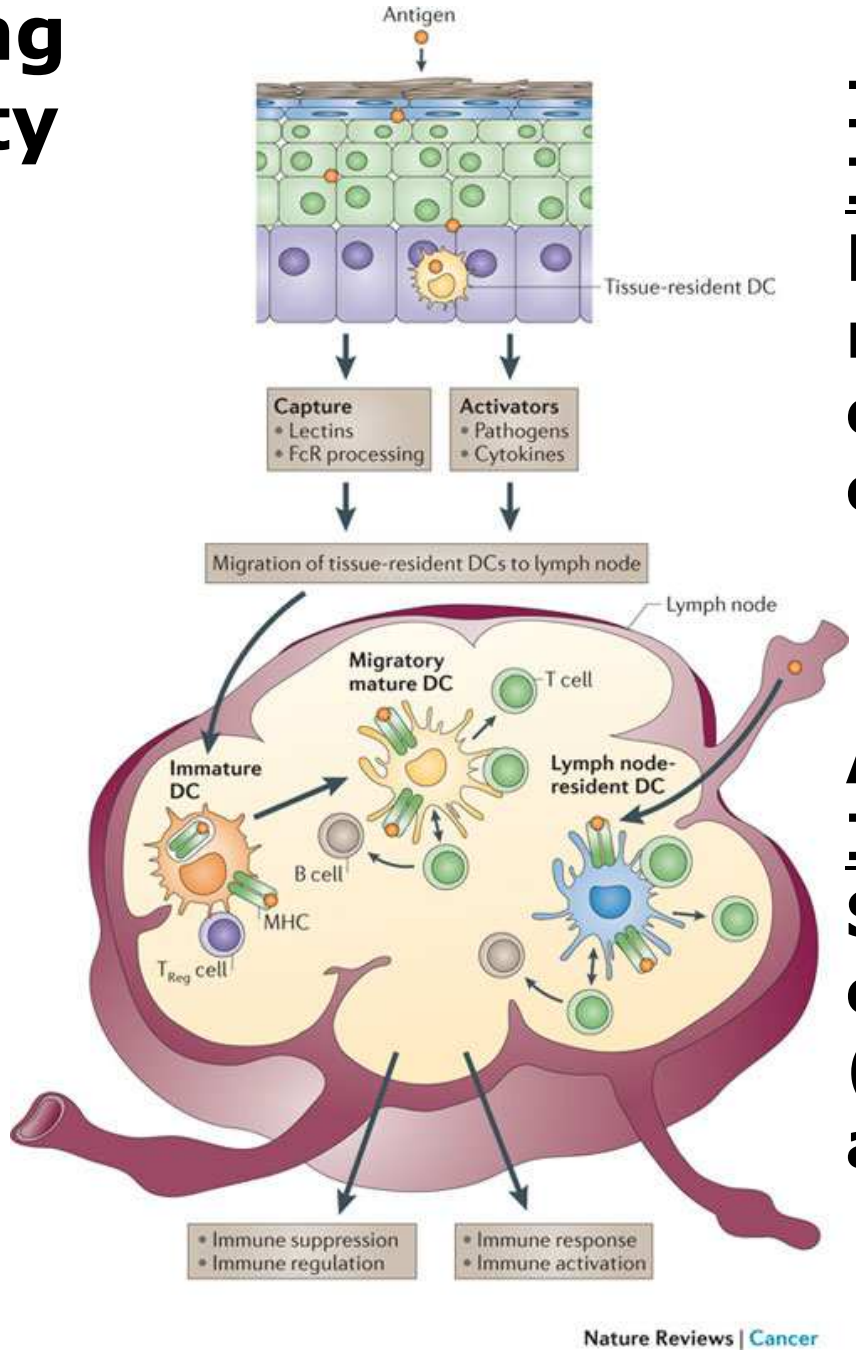
The following relationships exist related to this presentation:

No Relationships to Disclose related to this presentation

But in the interests of full disclosure, the following relationships exist (2014):

Advisory Board participation:
Dianippon Sumitomo Pharma, Astellas
NeoStem, Scientific Advisory Board member,

Launching immunity



Innate Immunity
Non-clonal receptors; endosomal and cytosolic sensors

Adaptive Immunity
Surface clonal receptors (TCRs BCRs); antigen specificity

Palucka & Banchereau; Nature Reviews Cancer 12, 265-277 (April 2012)

Dendritic Cells at the center of the immunologic universe

Sampling their environment

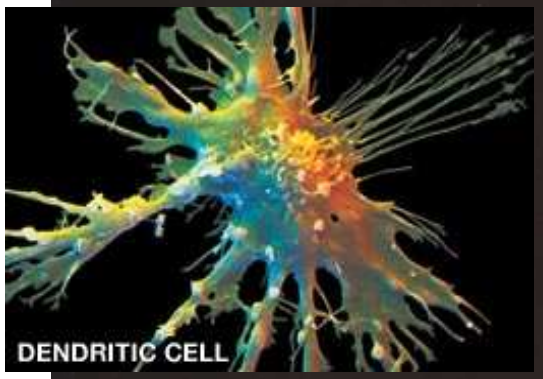
Sensing pathogens

Trafficking from the periphery to lymph nodes

Presenting antigen and shaping the adaptive immune response

Inhibiting unwanted responses (tolerance) and activating needed responses

Many different types of DC



Dendritic cells: Understanding immunogenicity

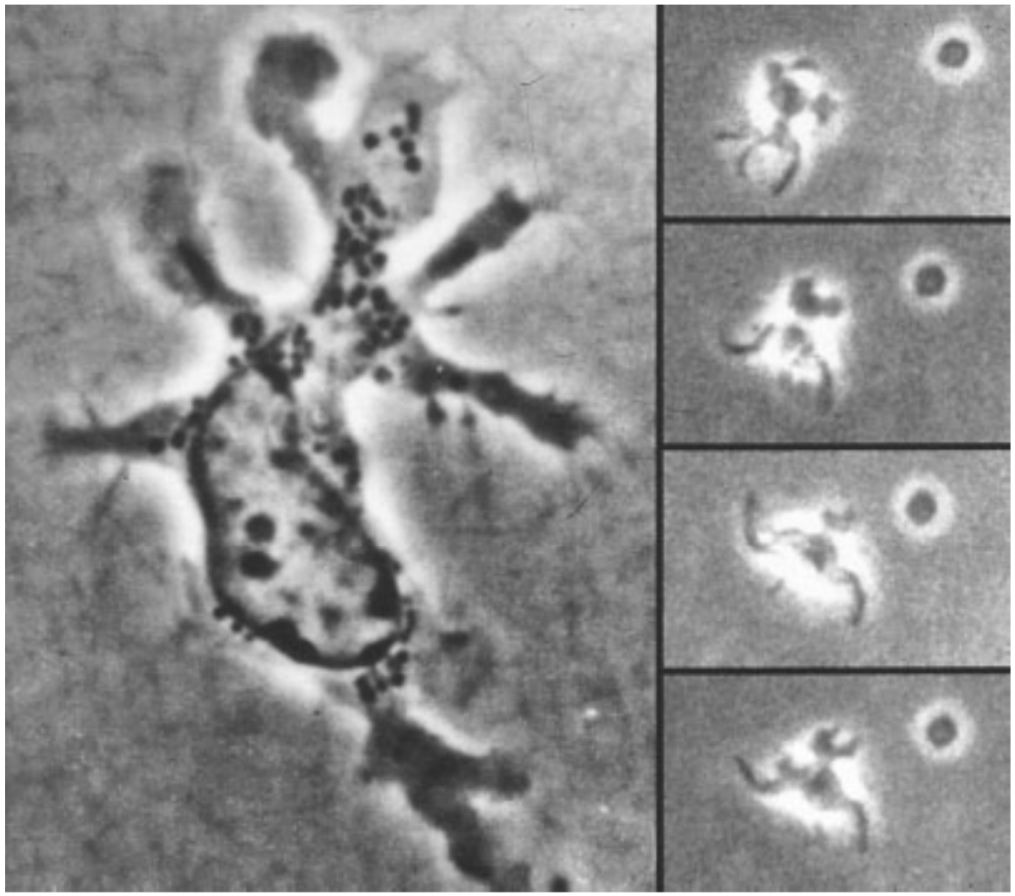


Figure 1. Phase contrast micrographs of dendritic cells. Left. A mouse spleen DC cultivated 24h in a plasma clot. Right. Four successive views of a living human blood DC cultivated in liquid medium to show the rapidly changing cell shape. The

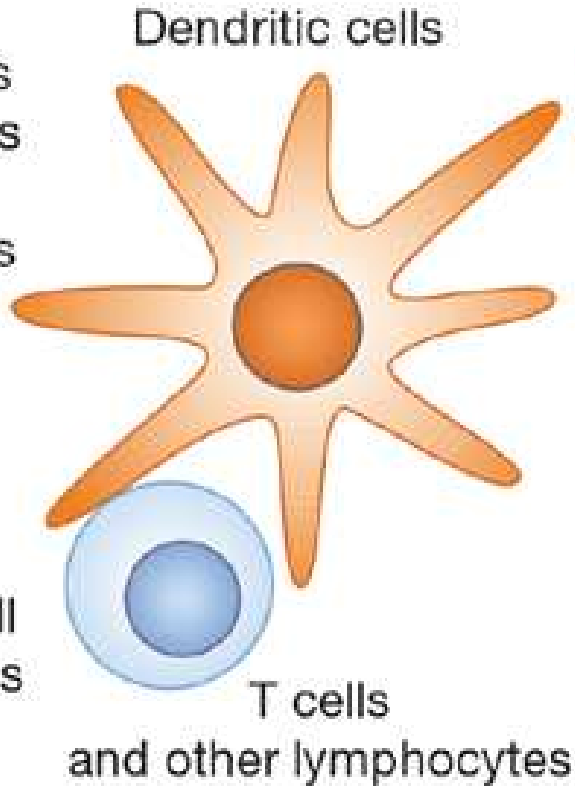
Ralph M. Steinman

Eur. J. Immunol. 2007. *37*: S53–60

Dendritic cells: Ralph M. Steinman, MD
2011 Nobel Prize in Medicine or Physiology

Dendritic Cells

Antigen uptake receptors and processing pathways for **presentation** of peptide–MHC complexes



Maturation or differentiation in response to microbial and other stimuli

Location at body surfaces and in the T-cell areas of lymphoid organs

Subsets with distinct pattern recognition receptors and functions

Steinman & Banchereau Nature 2007

Regulation

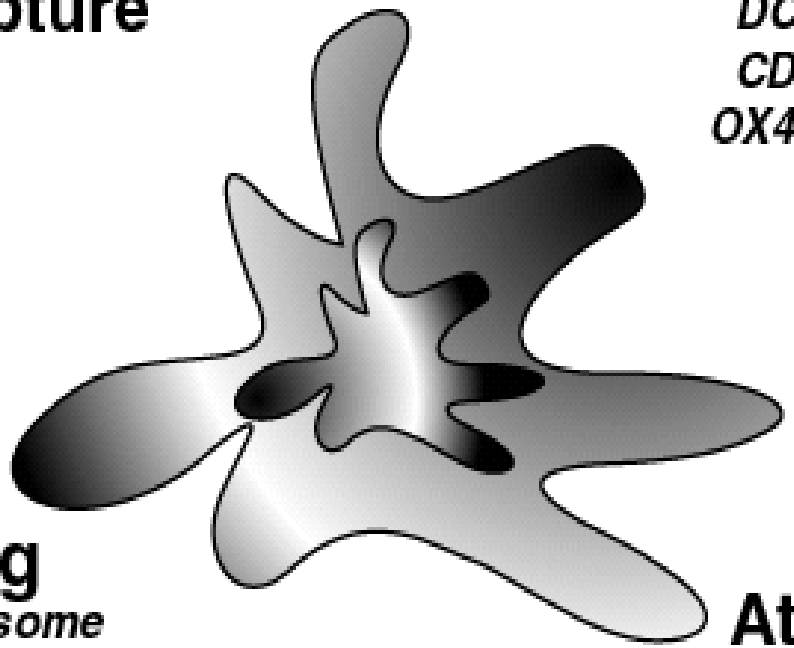
IFN-α, IL-12, IL-10
IL-18, IL-6

Costimulation

DC-SIGN, LFA, ICAMs
CD40, B7-1, B7-2, B7h
OX40-L, 41-BB, TRANCE
Blys

Recognition/Capture

Toll receptors
Mannose R
DEC205
Langerin
FcR, CD36, VNR
Aquaporins



Presentation

MHC class II, class I
CD1

Processing

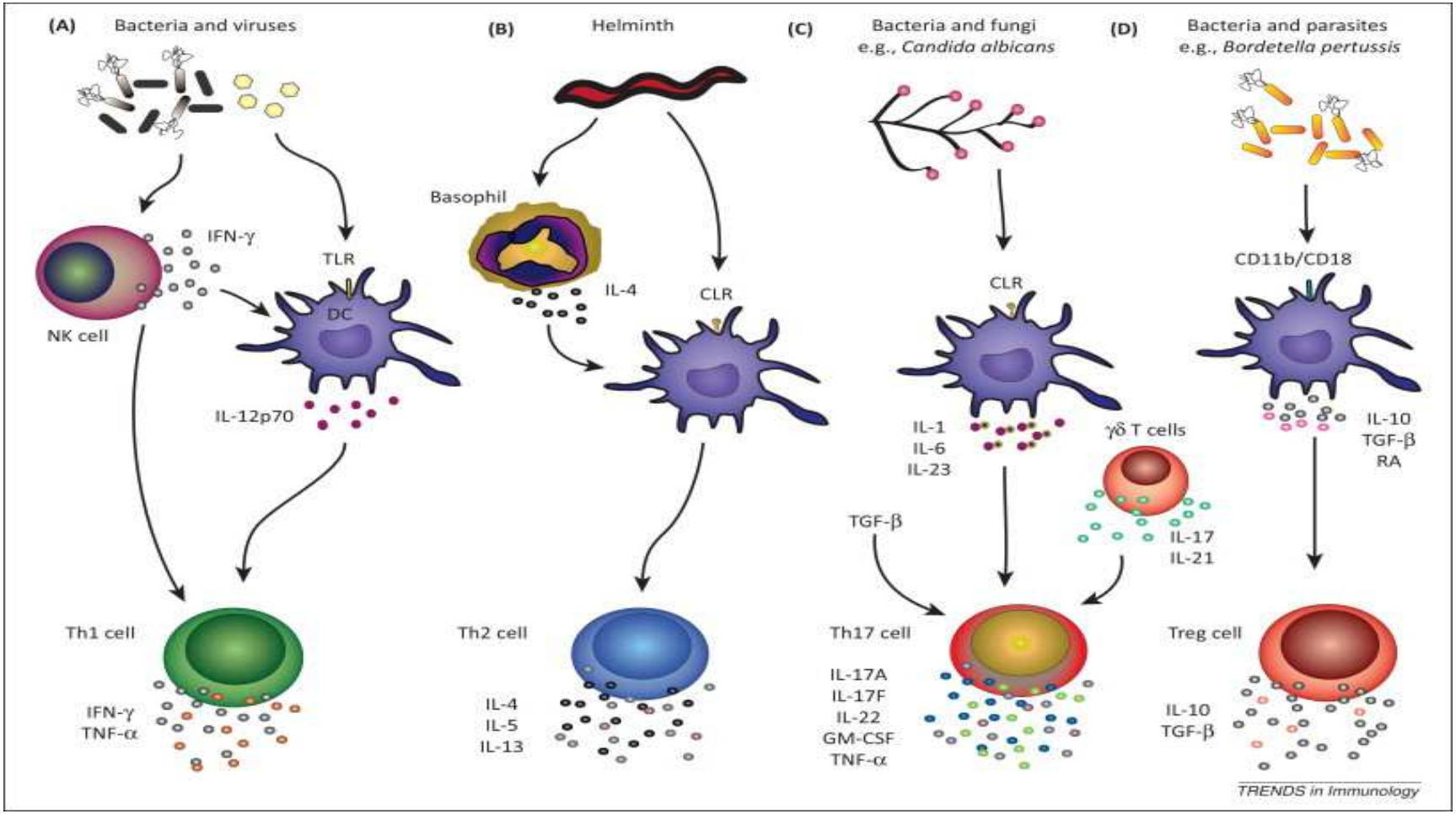
Endosome / Proteasome
Cystatin
DC-LAMP
Cathepsins

Attraction

DC-CK1
MDC, TARC
Fracktalkine
RANTES, MIP-1
MIP-3β
IL-8, IL15

Migration

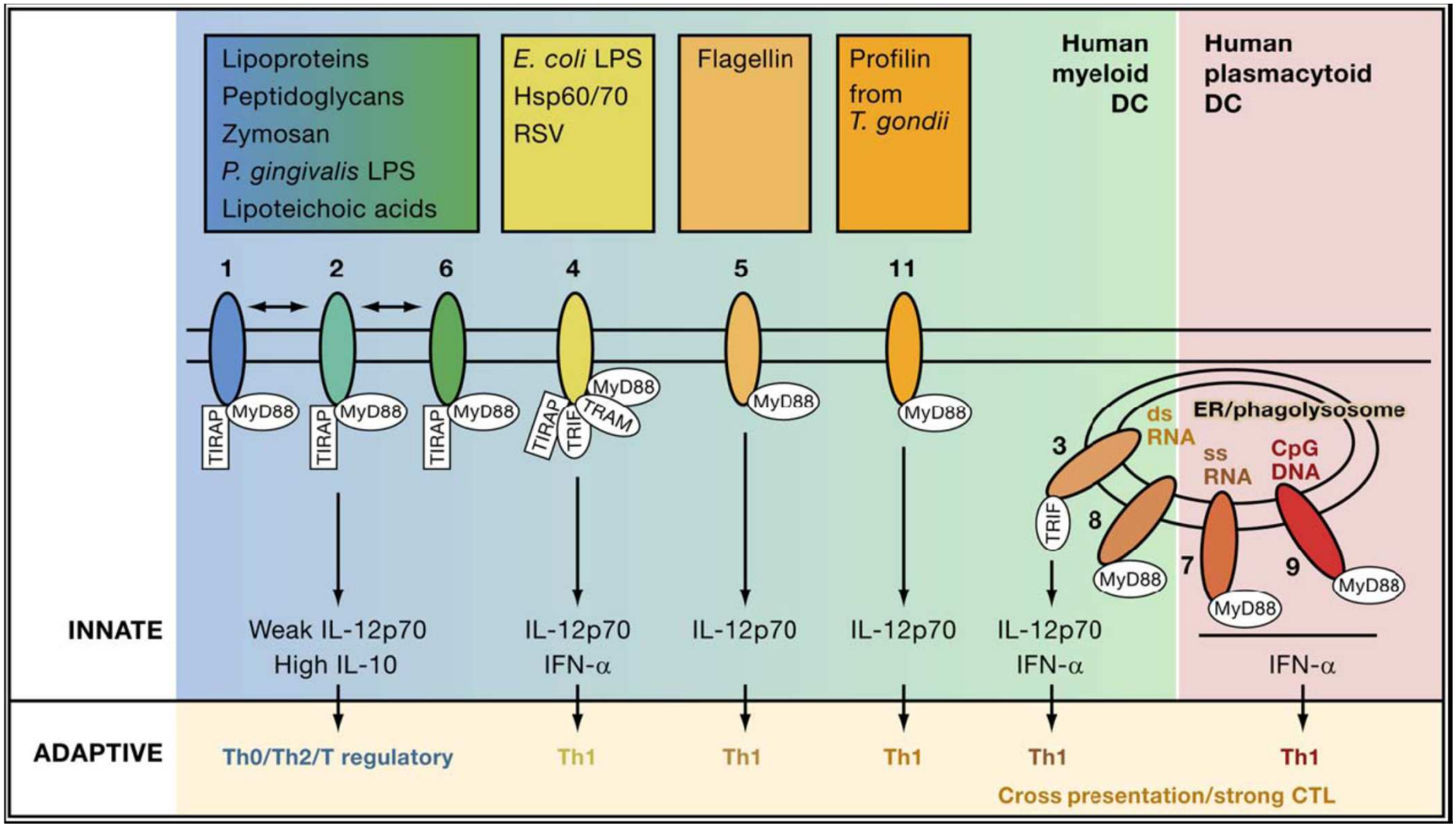
CCR5/MIP-1α
CCR6/MIP-3α, βdefensins
CCR7/6Ckine, MIP-3β
E-cadherin, CD44
CCR7/6Ckine, MIP-3β



<u>Pathogens direct the induction of Th cell subtypes via distinct effects on innate immune cells.</u> **(A)** TLRs recognise bacterial and viral PAMPs and drive IL-12p70 production by DCs. IFN-γ from innate immune cells such as NK cells augments the production of IL-12p70. **(B)** CLRs such as mannose receptor have been shown to recognise helminth-derived products such as the SEA Omega-1 glycoprotein which activates DCs to drive Th2 cell induction. In some systems Th2 cell induction is independent of IL-4, whereas in others cells such as TSLP-dependent basophils are required for optimal Th2 cell differentiation. **(C)** The CLR Dectin-1 recognises β glucans from several fungal species and initiates cytokine production by DCs which drives Th17 cell differentiation. The key differentiating factors for Th17 cell induction from naive T cells are IL-6, TGF-β, and IL-21, with IL-1 and IL-23 being required for activation and expansion of Th17 cells. **(D)** PAMPs from bacteria such as *Bordetalla pertussis* bind to CD11b/CD18 on DCs and stimulate IL-10 production, which in turn drives the induction of IL-10-producing Treg cells.

Walsh and Mills, **Dendritic cells and other innate determinants of T helper cell polarisation.** 2013 Trends Immunology, V34, #11

TRIGGERING DISTINCT TLRs ON DCs ELICITS DIFFERENT CYTOKINE PROFILES AND DIFFERENT IMMUNE RESPONSES

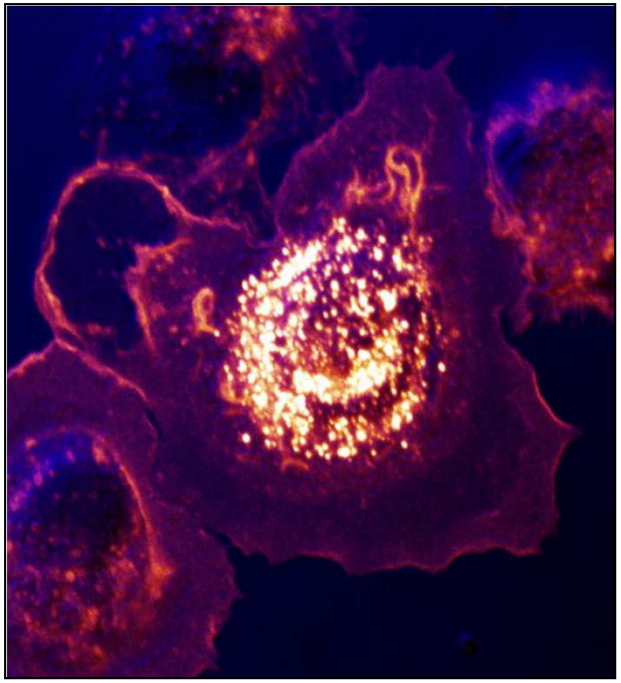


Pulendran & Ahmed. 2006 *Cell*

DENDRITIC CELL MATURATION

The control point of cellular immunity

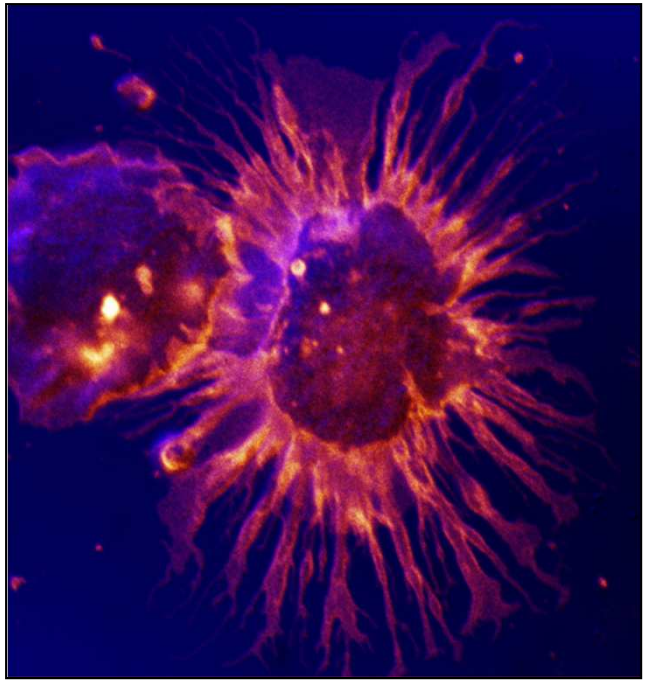
Microbial Products/Adjuvants:
TLR, NOD and lectin ligands
LPS, DNA, RNA



Tissue damage:
Uric acid, HSPs

Cells of innate immunity
pDC, NK, NK T, Neutrophils
IFN, TNF, GM-CSF

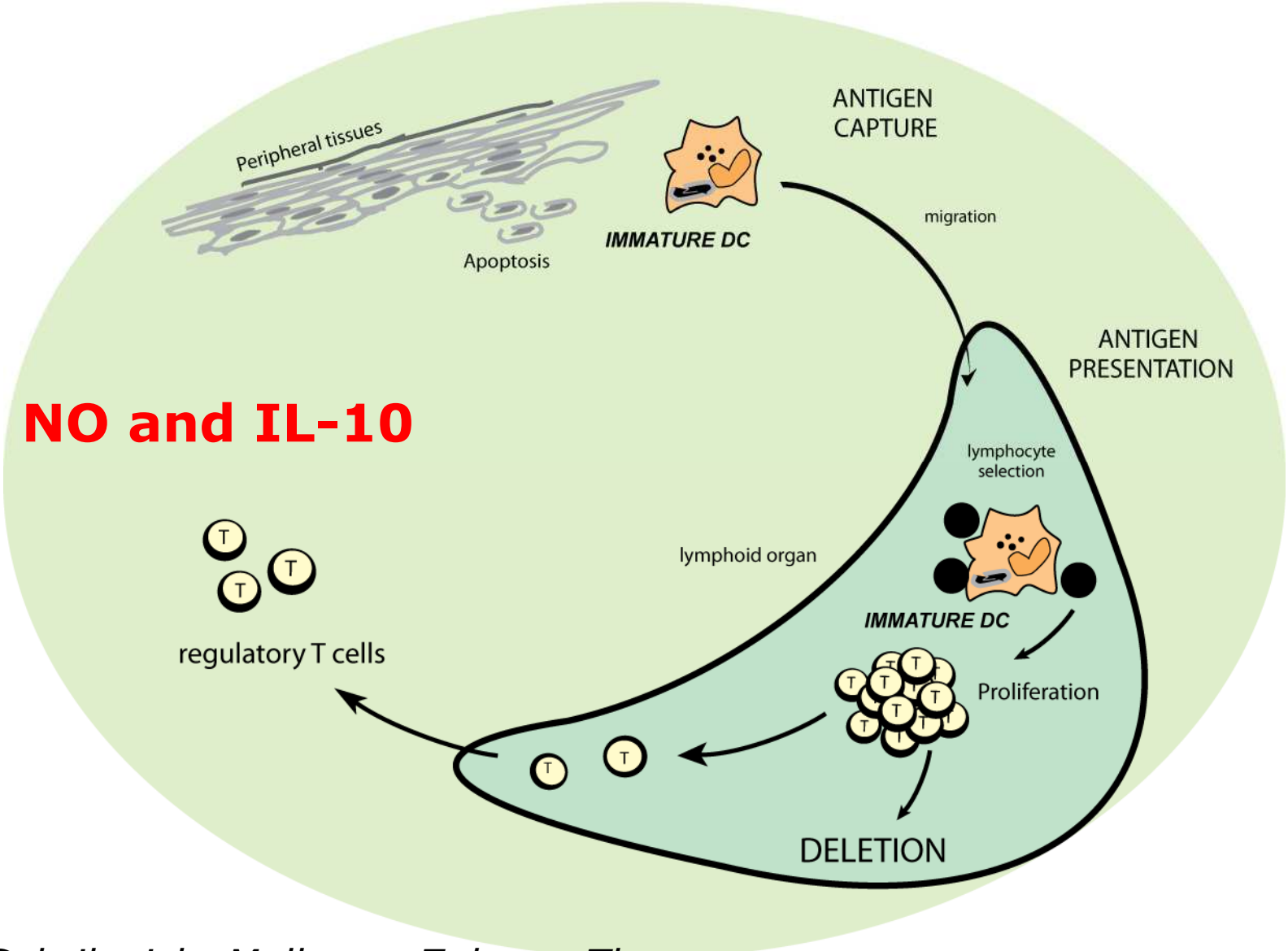
Cells of adaptive immunity
T and B cells
CD40L, RANK



Immature DC → **Mature DC**

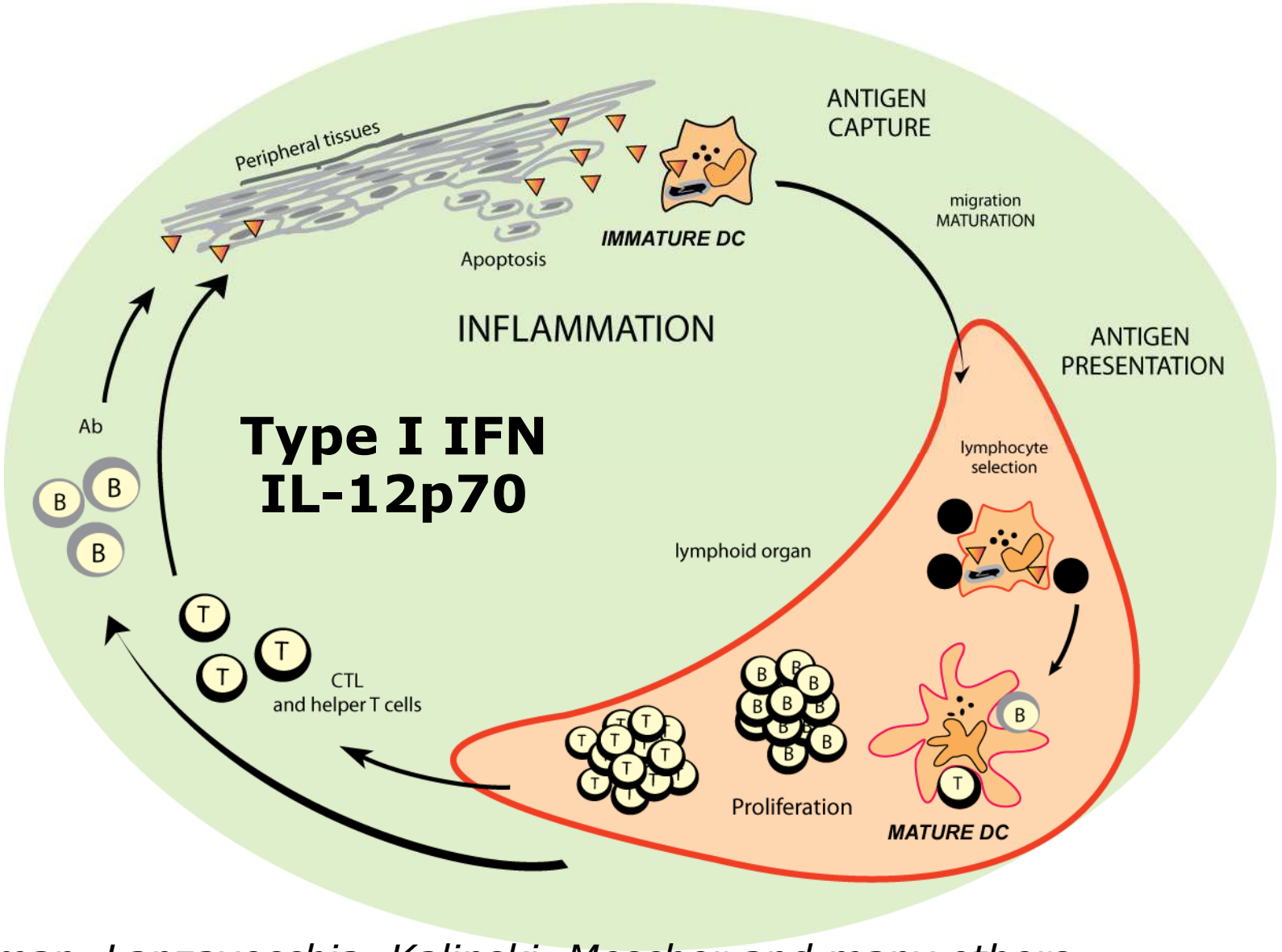
Steinman & Mellman

IMMATURE DENDRITIC CELLS ALLOW PERIPHERAL TOLERANCE



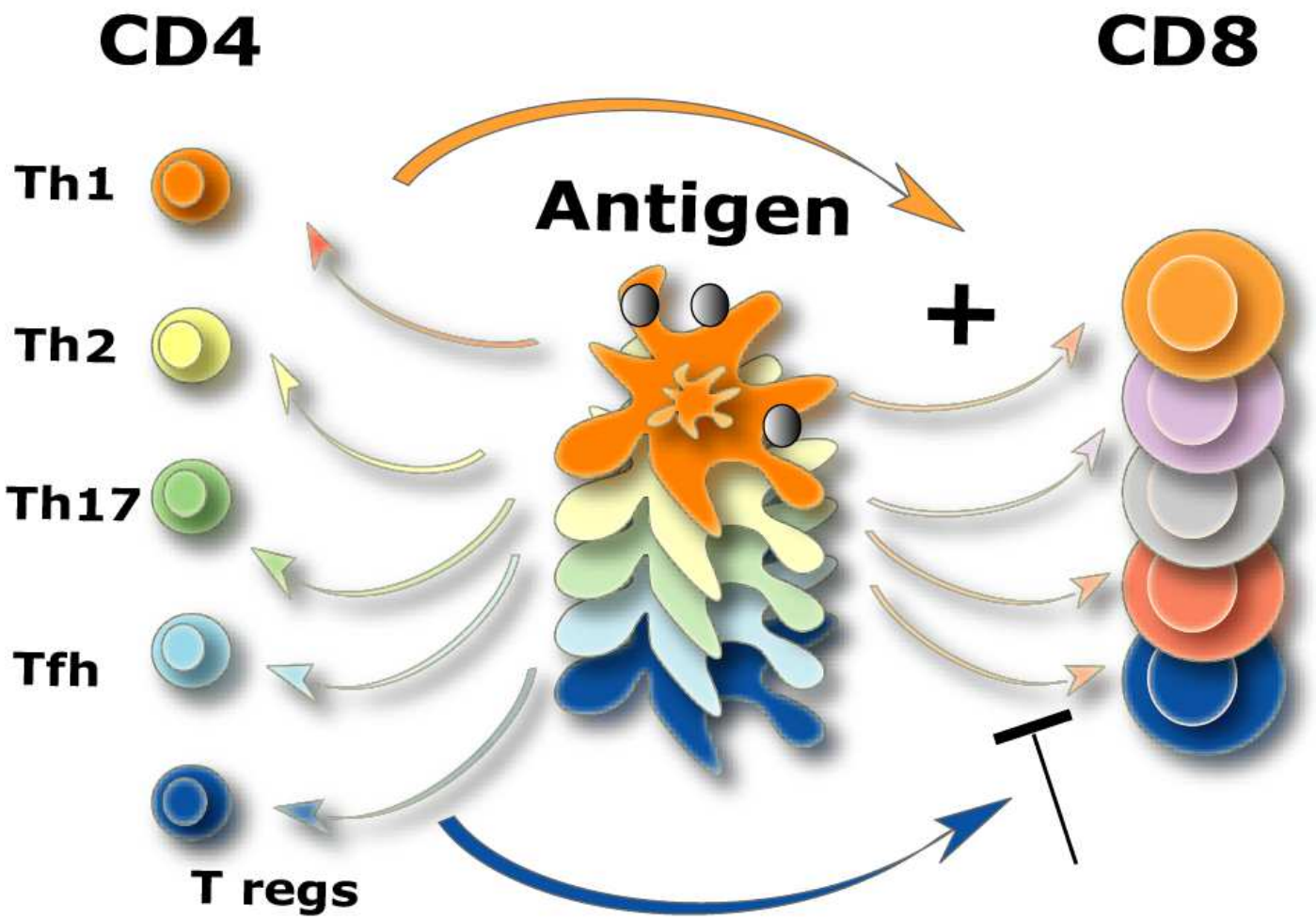
Cao, Gabrilovich, Mellman, Zahner, Thomson

MATURE DENDRITIC CELLS ALLOW IMMUNITY



Steinman, Lanzavecchia, Kalinski, Mescher and many others

The complexity of eliciting immune response: Which antigens, which dendritic cells, which immune responses?

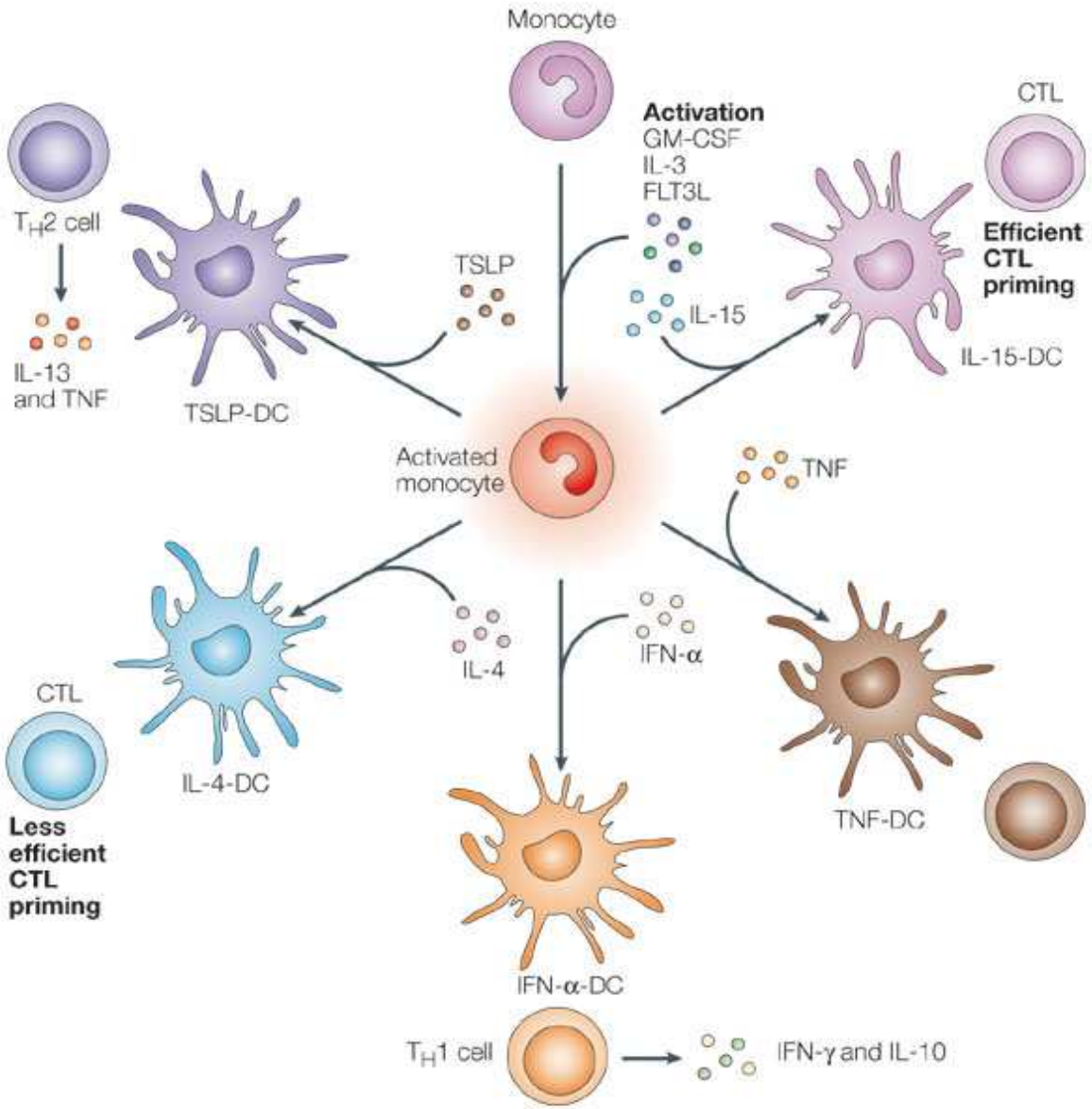


The Human DC Compartment

	pDC	BDCA1^{+} (CD1c)$^{+}$	BDCA3^{+} (CD141)$^{+}$	LC	CD14^{+}	CD1a^{+}
Phenotype:	Lin^{-} HLA-DR^{+} CD11c^{low} CD1a^{-} CD123^{hi} BDCA2^{+} BDCA4^{+}	Lin^{-} HLA-DR^{+} CD11c^{+} CD1a^{-} BDCA1^{+} BDCA3$^{+/-}$ CD11b^{low}	Lin^{-} HLA-DR^{+} CD11c^{+} CD1a^{-} BDCA1^{-} BDCA3^{+} CD11b^{low} CD141^{+} Necl2^{+} Xcr1^{+} Clec9a^{+} Dec205^{hi}	Lin^{-} HLA-DR^{+} CD11c^{+} CD1a^{+} CD14^{-} BDCA1^{+} Langerin^{+} EpCAM^{+} Sirpα^{+} CD11b$^{+/-}$ E-cadherin^{+}	Lin^{-} HLA-DR^{+} CD11c^{+} CD1a^{-} CD14^{+} BDCA1^{+} Langerin^{-} EpCAM^{-} DC-SIGN^{+} FXIIIa^{-} CD163^{-}	Lin^{-} HLA-DR^{+} CD11c^{+} CD1a^{+} CD14^{-} BDCA1^{+} Langerin^{-} EpCAM^{-} Sirpα^{+} CD11b^{hi}
PRRs:	TLR1^{+}, TLR2^{-}, TLR3^{-}, TLR4^{-}, TLR6^{+}, TLR7^{+}, TLR8^{-}, TLR9^{+}	ND	TLR1^{+}, TLR2^{+}, TLR3^{+}, TLR4^{-}, TLR6^{+}, TLR7^{-}, TLR8^{+}, TLR9^{-}	TLR1^{+}, TLR2^{+}, TLR3^{lo}, TLR4^{-}, TLR6^{+}, TLR7^{-}, TLR8^{-}, TLR9^{-}	ND	ND
Murine equivalent:	pDC	cDC	CD8^{+} cDC	LC	ND	Dermal DC
Location:	Blood and lymphoid tissue			Epidermis	Dermis	Cutaneous tissue

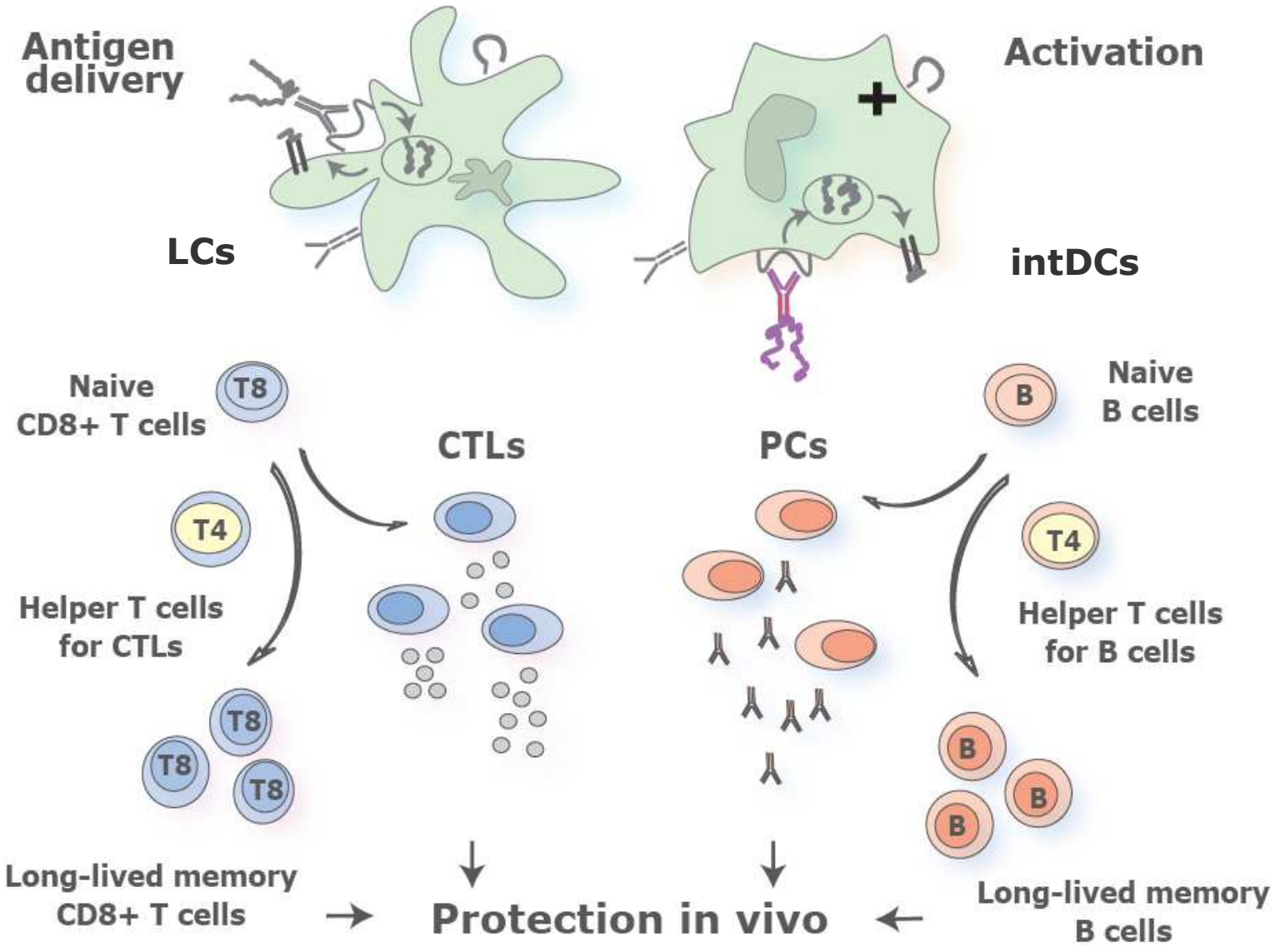
Merad et al. Annual Review of Immunology 2013

Multiple monocyte-derived DCs



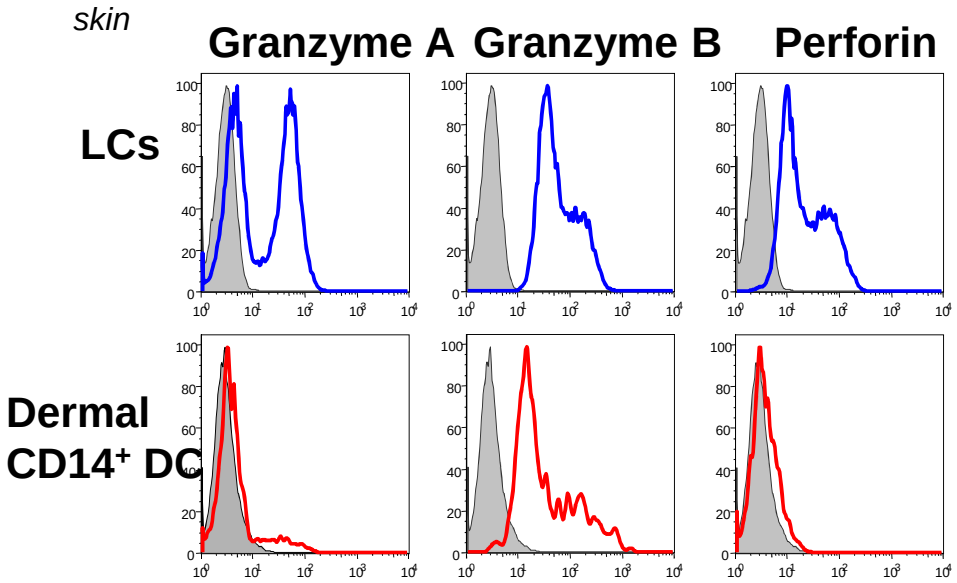
Banchereau and Palucka Nat Rev Immunol 2005

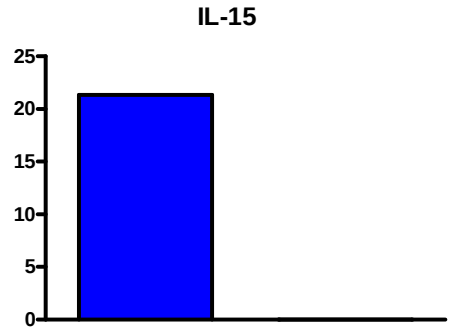
LANGERHANS CELLS PREFERENTIALLY CONTROL CELLULAR IMMUNITY
DERMAL DC PREFERENTIALLY CONTROL HUMORAL IMMUNITY

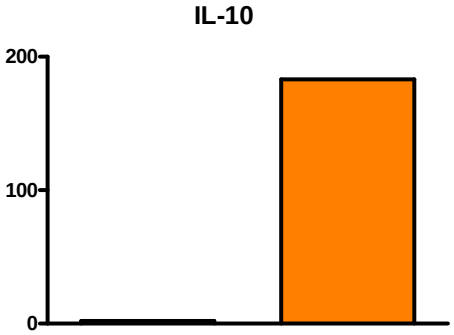


Klechevsky, Ueno et al Immunity 2008

LCs efficiently prime effector CD8$^+$ T cells

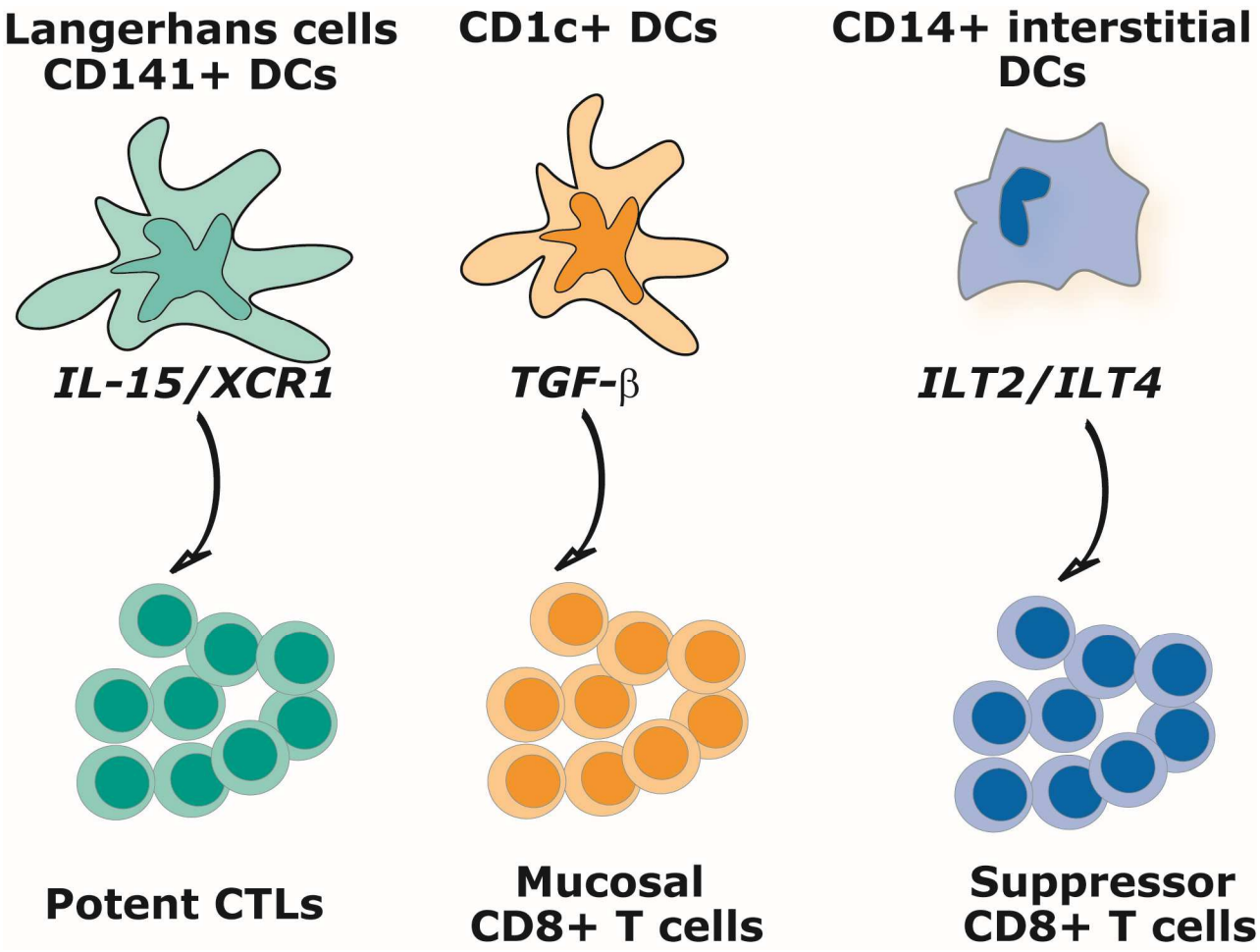






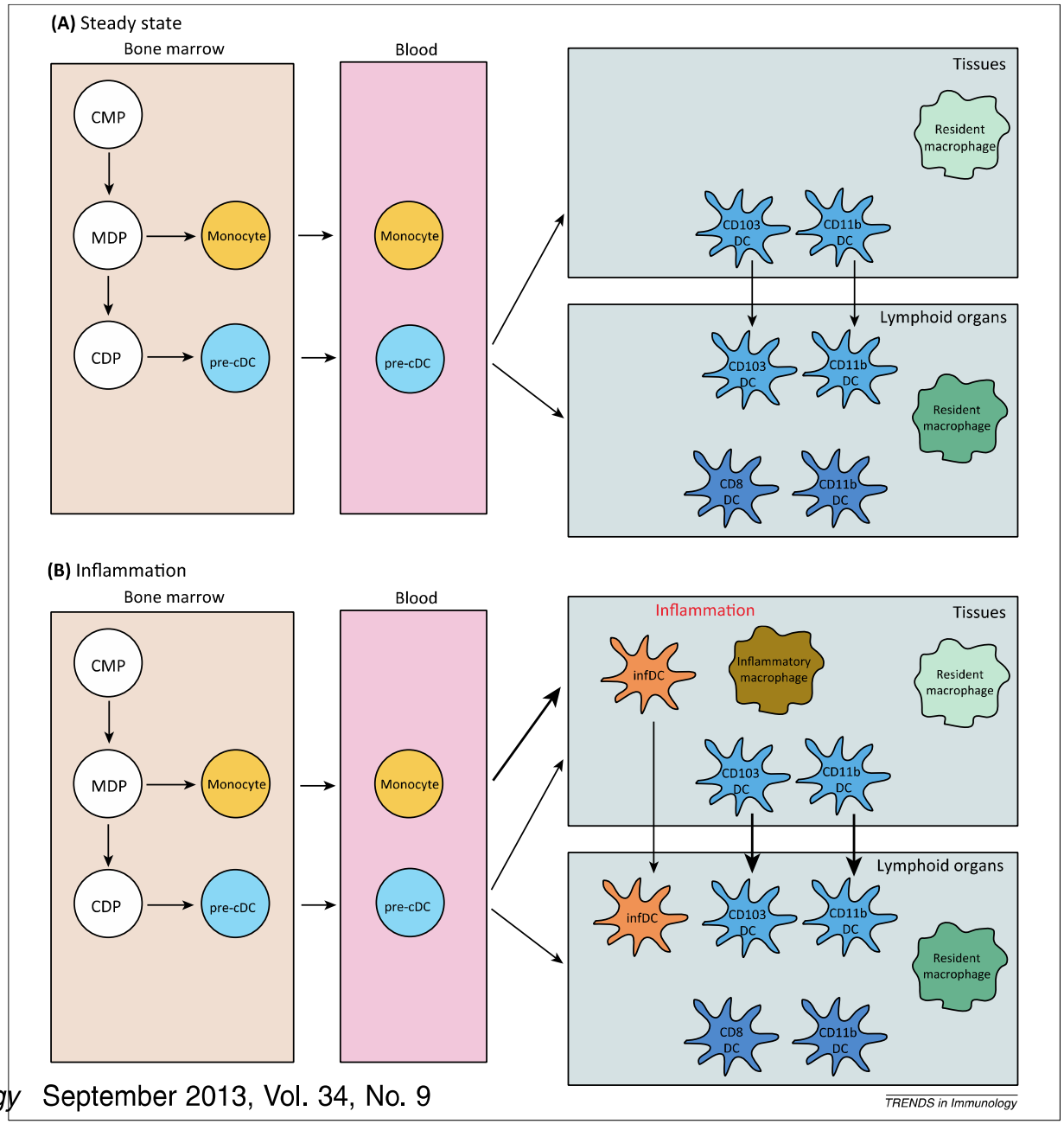
Klechevsky et al: Immunity, 2008
Banchereau,Klechevsky: Blood, 2012

Distinct DC subsets elicit CD8^{+} T cells with distinct phenotypes



Palucka & Banchereau Curr Opin Immunol 2013

Human DCs in vivo



Amigorena et al
Immunity 2013

Trends in Immunology September 2013, Vol. 34, No. 9

Immunological goals of vaccination

Prophylactic Vaccines:

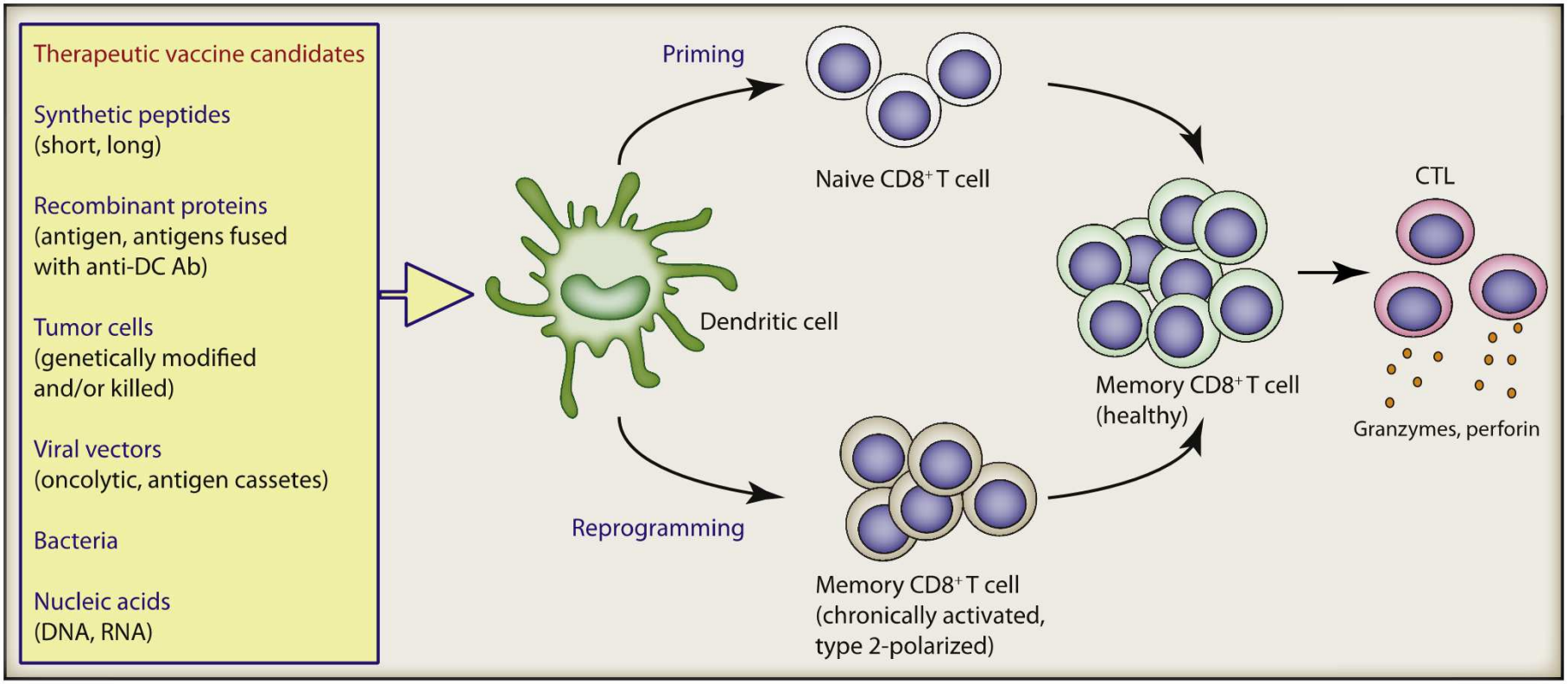
- Priming, i.e. generation of new immune response
- Boosting immune memory

Therapeutic Vaccines:

- Priming
- Re-programming pathogenic immune memory to protective one
- Generating $CD8^+$ T cells able to kill cancer

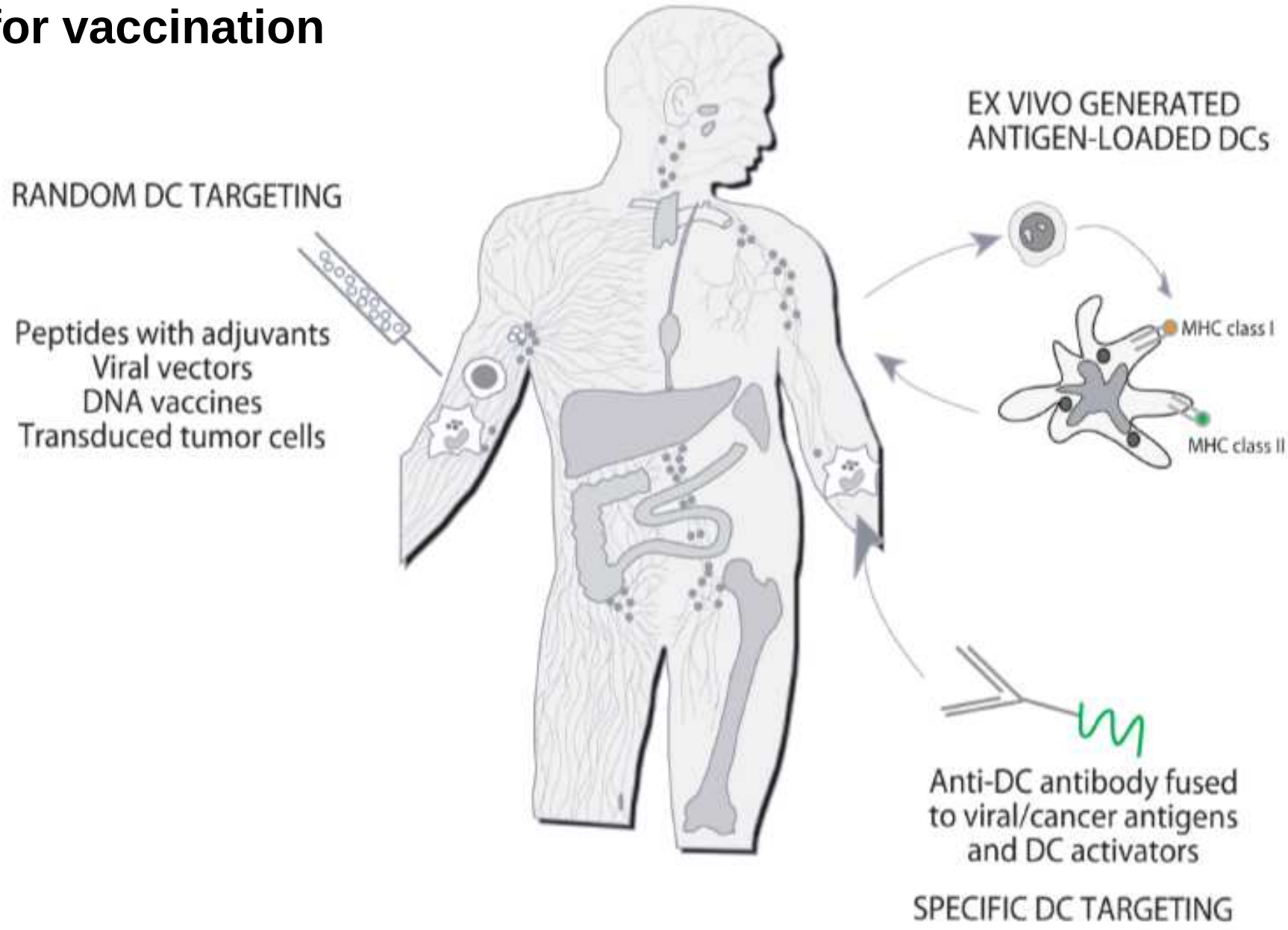
Palucka & Banchereau Immunity 2013

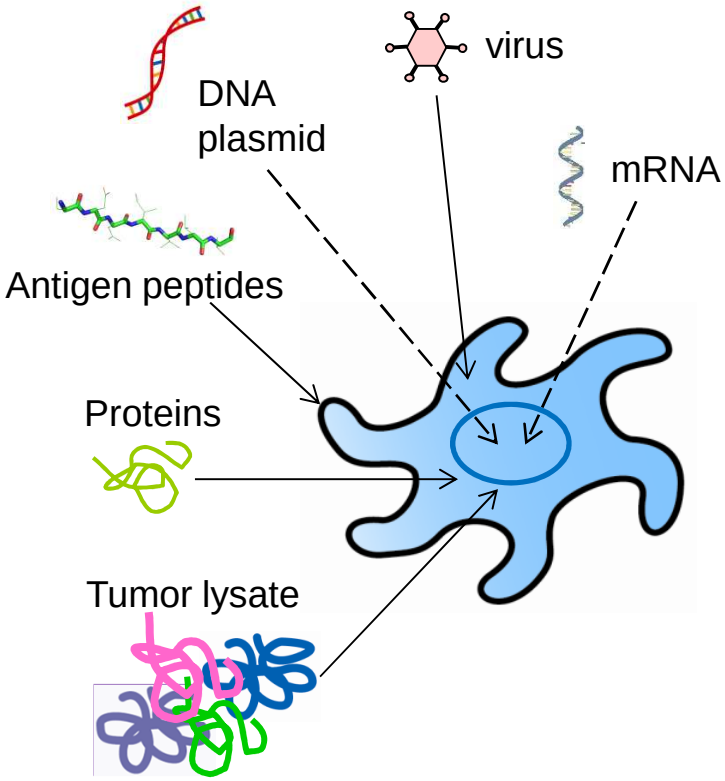
Therapeutic cancer vaccines act via dendritic cells

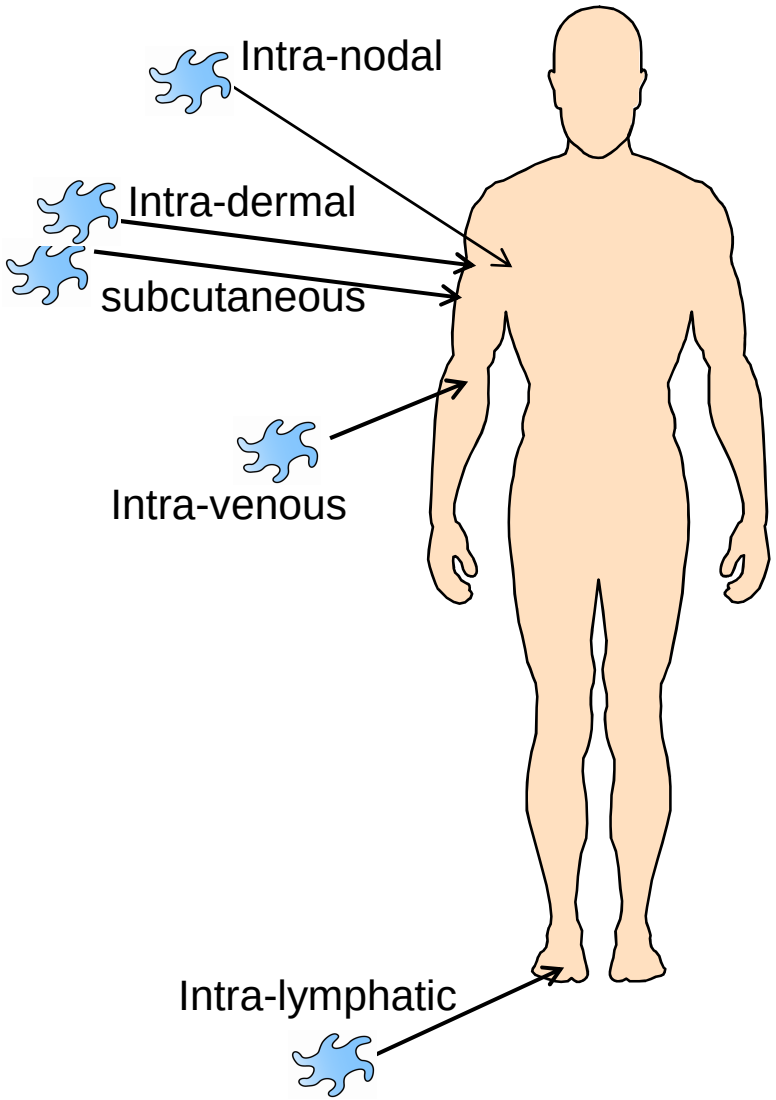


Palucka & Banchereau, Immunity, 2013

Dendritic cells as targets for vaccination




virus
DNA
plasmid
mRNA
Antigen peptides
Proteins
Tumor lysate


Intra-nodal
Intra-dermal
subcutaneous
Intra-venous
Intra-lymphatic

Dendritic Cell Vaccines

Successes: >7% clinical response rate in late stage patients

Failures: <6% responses rate that was similar to chemotherapy in a randomized trial; other trials without significant clinical responses

Current questions:

- **Dose** (10e5 minimum? 10e8 maximum feasible?)
- **Route** (i.d. > i.v.? i.n accuracy? i.lymphatic?)
- **Culture conditions** (can we do better than 6 days in GM+IL-4?)
- **Maturation conditions** (TLRs, cocktails…)
- **Antigen loading** (peptides, proteins, lysates, allo, autologous…)
- **Potency Assay** (IL-12p70? IL-12/10? Phenotype? Transcriptome?)

Cellular Product minimal safety, purity and identity tests.

The following is an example of the specific release tests which are required by the FDA for early phase trials involving autologous, *in vitro* manipulated cellular products (in this example, DC). This example also shows the identity/purity testing chosen for this type of product, and the candidate potency test being performed.

Viability: The cells are counted by microscopic observation on a hemacytometer, and a differential count (DC vs. lymphocytes) is obtained using trypan blue dye. Minimum 70% viability.

Purity: The DC must express MHC class II and CD86 by flow cytometry in a minimum of 70% of the cells. Additional phenotyping (MHC class I, CD80, CD83, CCR7, others) is performed to fully characterize the DC, and is for research proposes.

Sterility: DC are tested by bacterial (aerobic and anaerobic) and fungal cultures at the Clinical Microbiology Laboratory. Final results of the microbial cultures are available in 14 days. Prior to release of the DC for vaccine use, a standard gram stain is performed and must be negative for the presence of microorganisms.

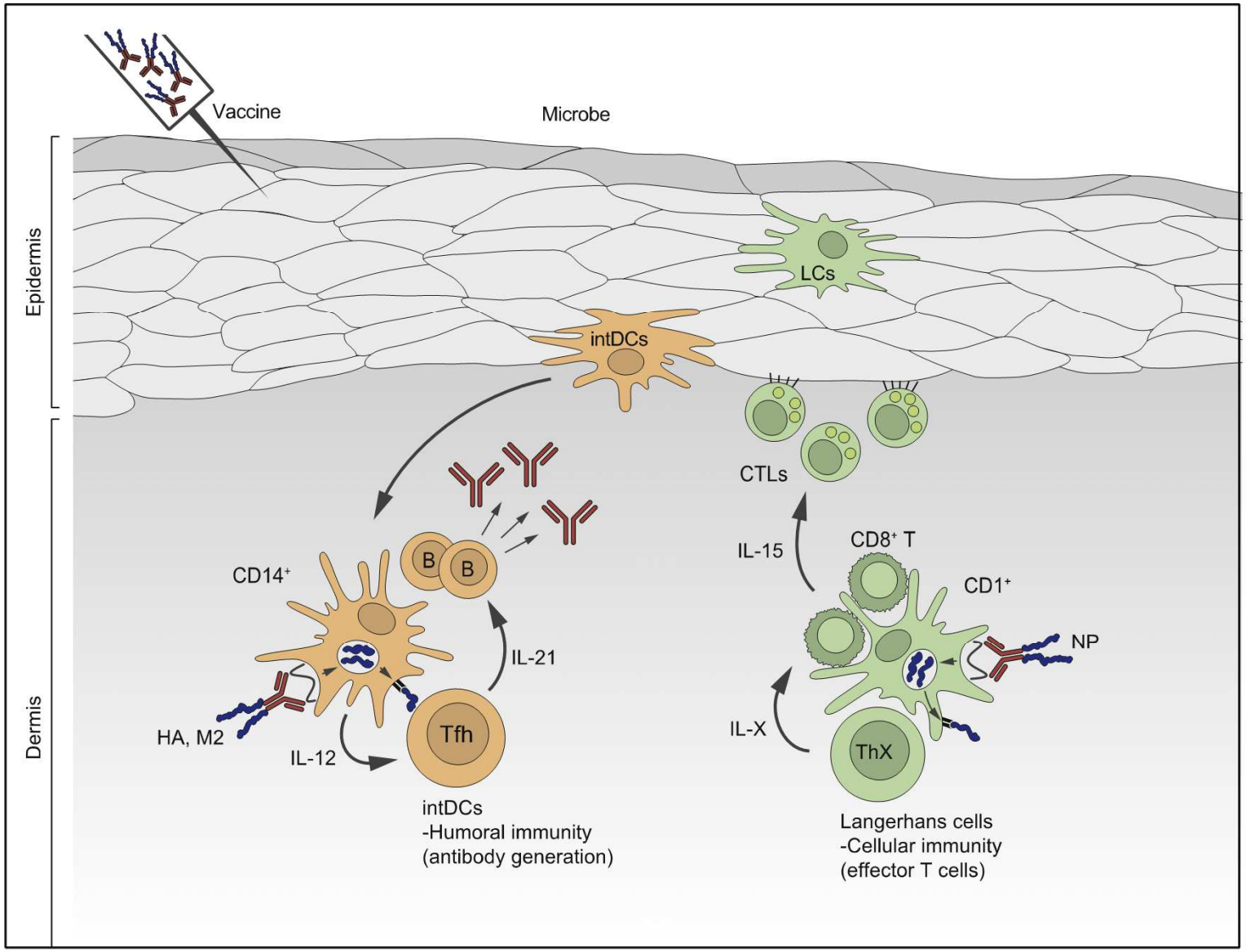
Mycoplasma testing of cell suspensions (not supernatants) is performed using a rapid detection system, based on nucleic acid hybridization or by PCR. The cell preparation must be negative for mycoplasma.

Endotoxin testing is performed on the cell culture at the time of harvest and prior to release of the final product. The acceptable endotoxin level is <5 EU/kg of body weight per dose. For intrathecally-administered cells, an upper limit of acceptance criterion is 0.2 EU/kg body weight/hour.

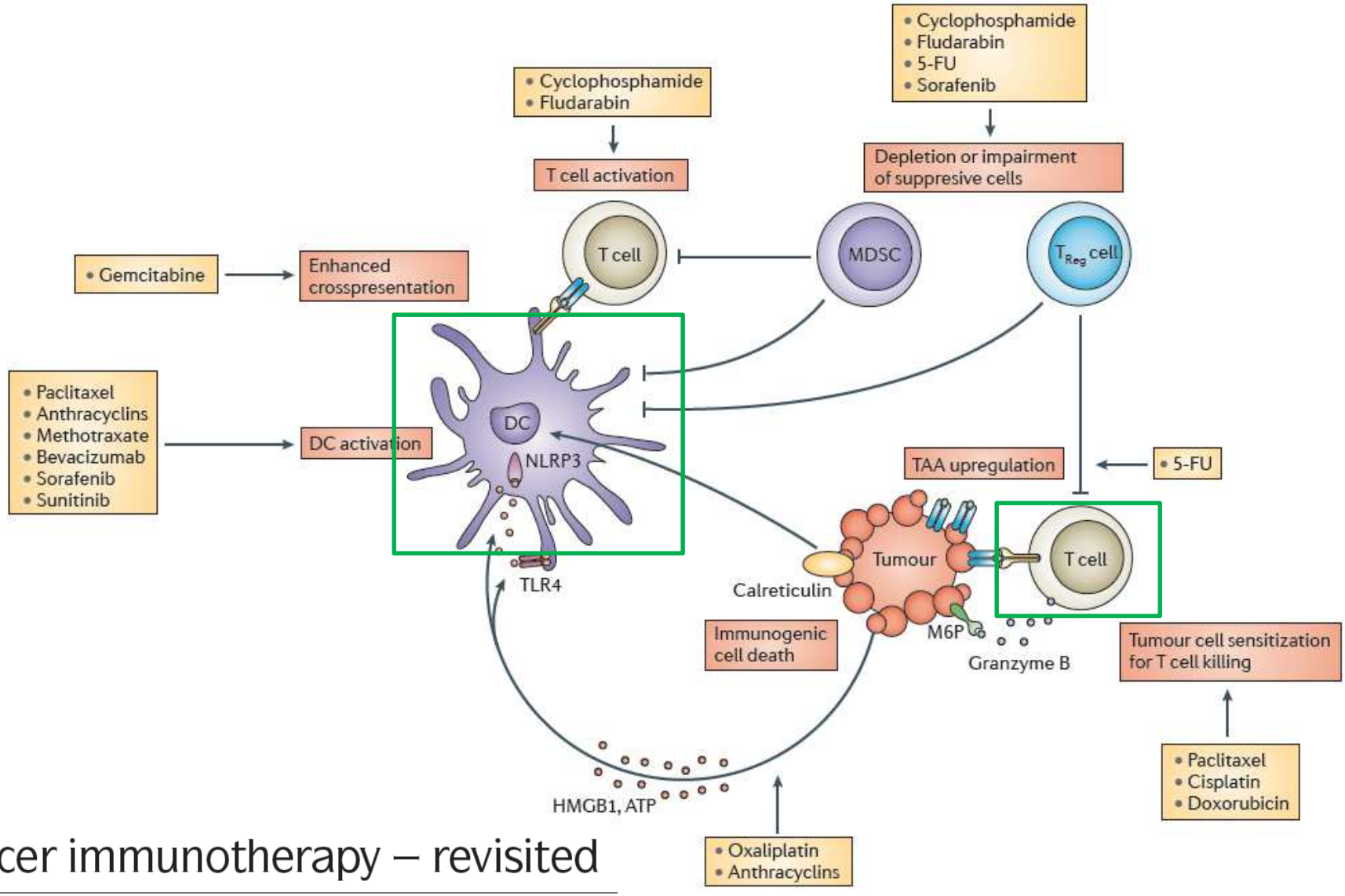
Potency: To define a measure of potency for the DC, we determine their ability to produce IL-12p70 and IL-10 by Luminex assay (25). This test is performed batched, with and without activation by CD40L and/or LPS, and is available several weeks after vaccine injection. Data will be correlated with measures of DC phenotype and biological activity such as antigen presentation and clinical outcome (88).

Additionally, a 0.5 ml sample of the final DC preparation from each vaccination time is cryopreserved for possible ancillary testing in the future. These samples are stored a minimum of one year after vaccine administration.

Directing the immune response: Targeting antigens to specific DC subsets



Chemotherapy and targeted therapy meet immunology: immuno-oncology



Cancer immunotherapy – revisited

W. Joost Lesterhuis, John B. A. G. Haanen‡ and Cornelis J. A. Punt**

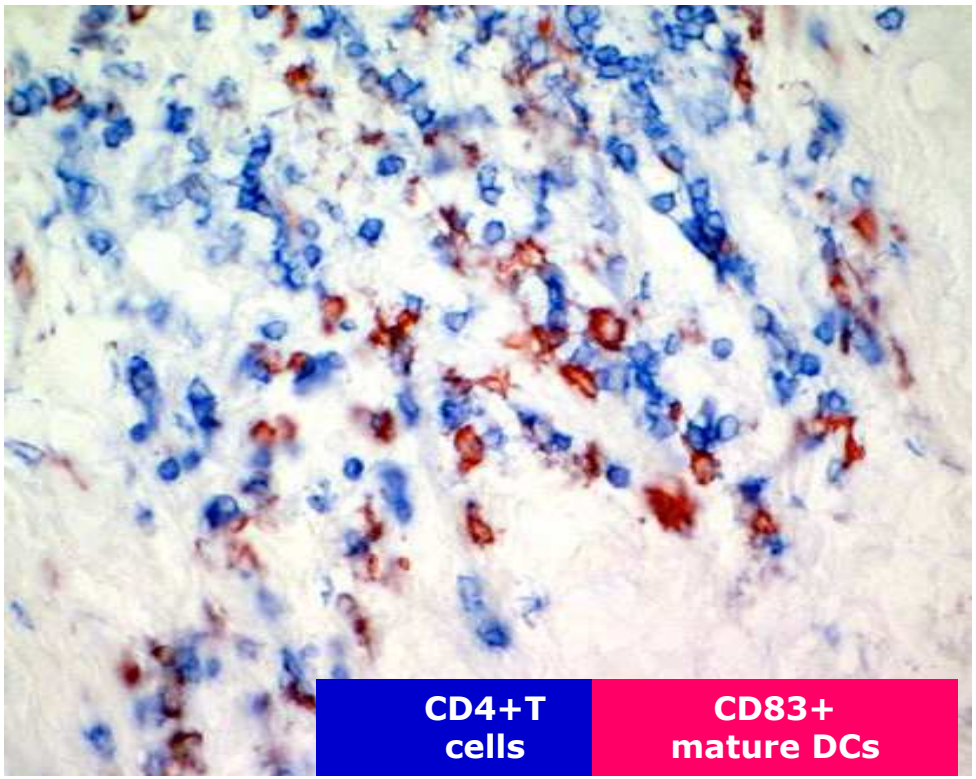
NATURE REVIEWS | DRUG DISCOVERY VOLUME 10 | AUGUST 2011

J. Exp. Med. © The Rockefeller University Press
Volume 190, Number 10, November 15, 1999
http://www.jem.org

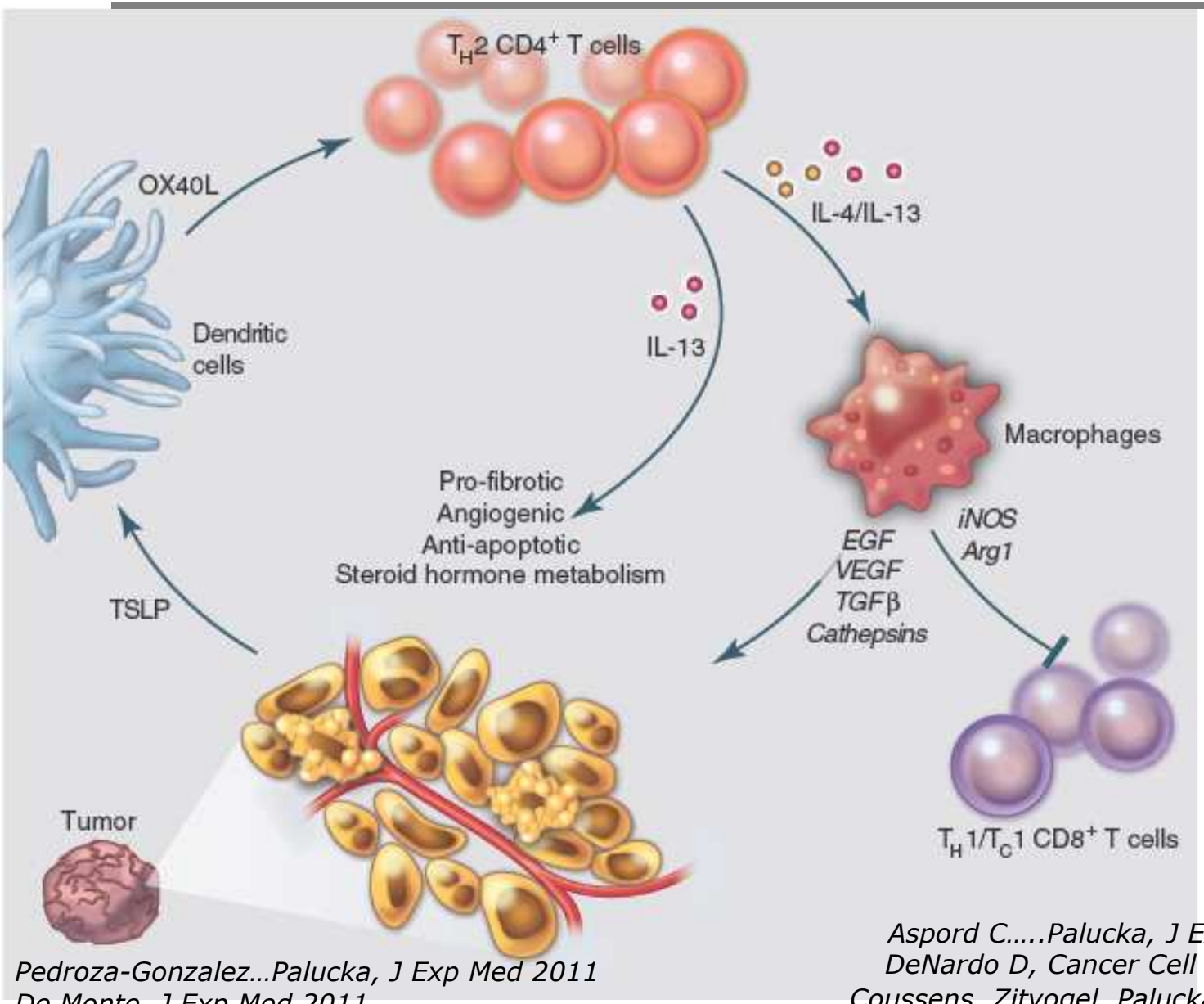
In Breast Carcinoma Tissue, Immature Dendritic Cells Reside within the Tumor, whereas Mature Dendritic Cells Are Located in Peritumoral Areas

By Diana Bell,* Pascale Chomarat,* Denise Broyles,* George Netto,* Ghada Moumneh Harb,* Serge Lebecque,‡ Jenny Valladeau,‡ Jean Davoust,* Karolina A. Palucka,* and Jacques Banchereau*

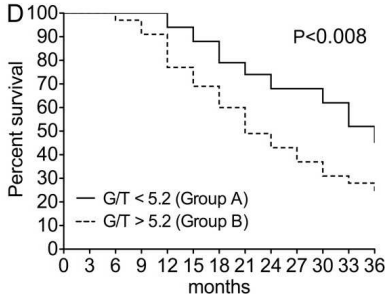
*From the *Baylor Institute for Immunology Research and Department of Pathology, Baylor University Medical Center, Dallas, Texas 75204; and ‡Schering-Plough Laboratory for Immunological Research, 69571 Dardilly, France*



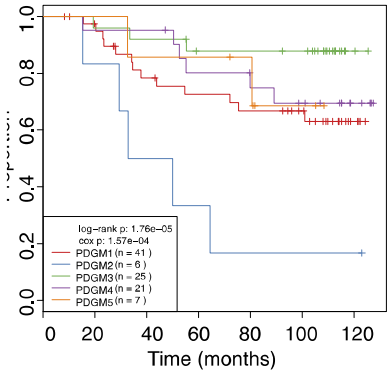
Pro-tumor Th2 inflammation in breast and pancreatic cancer



Pedroza-Gonzalez…Palucka, J Exp Med 2011
De Monte, J Exp Med 2011



GATA-3/T-bet ratio in pancreatic tumors De Monte JEM 2011

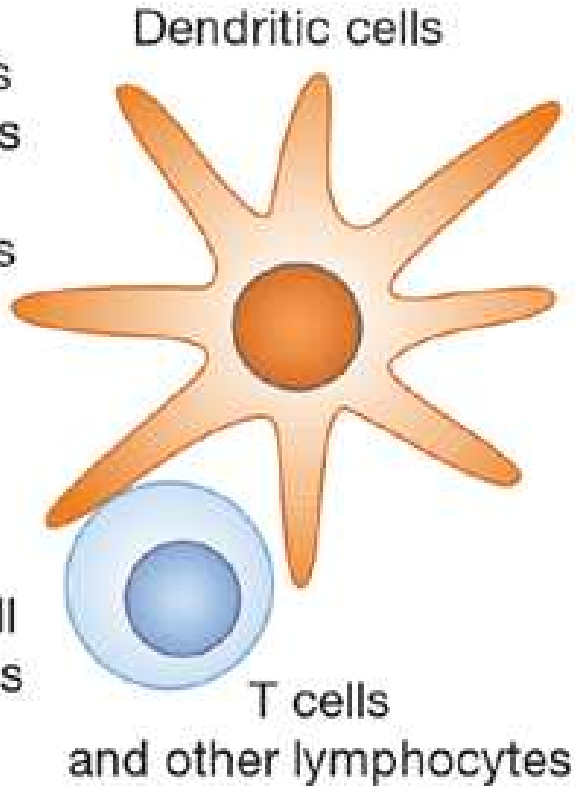


Low Th1/Th2 ratio in breast cancer Kristensen PNAS 2012

Aspord C…..Palucka, J Exp Med 2007
DeNardo D, Cancer Cell 2009
Coussens, Zitvogel, Palucka, Science 2013

Dendritic Cells

Antigen uptake receptors and processing pathways for **presentation** of peptide–MHC complexes



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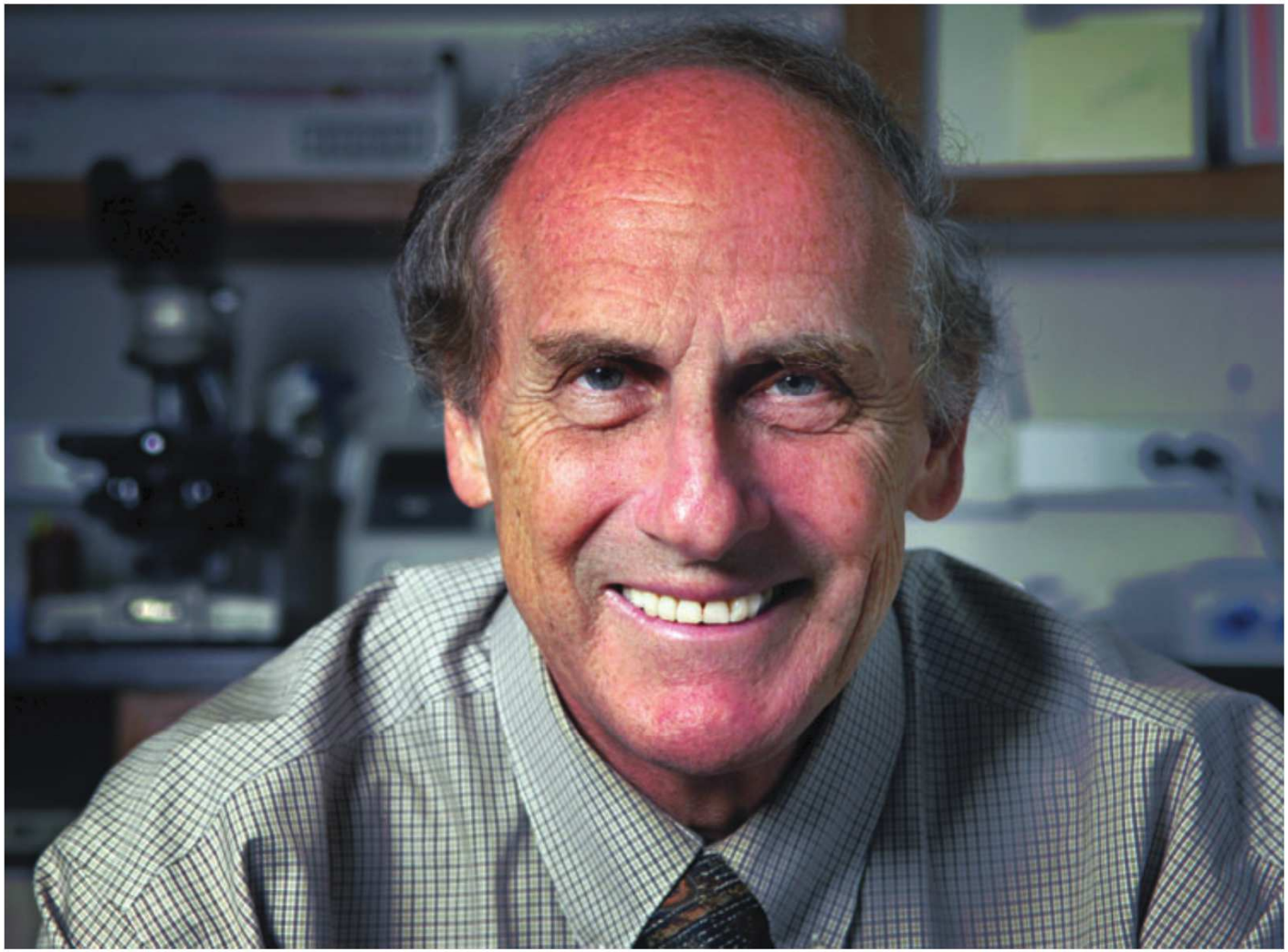
Steinman & Banchereau Nature 2007



Ralph M. Steinman

January 14, 1943 – September 30, 2011