Cells of Immunity – II

(Adaptive cells - APCs)

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CELLS OF ADAPTIVE IMMUNITY

- **LYMPHOCYTES**
  - **T CELLS**
  - **B CELLS**
  - **NK CELLS**
- **ANTIGEN PRESENTING CELLS**
  - **DENDRITIC CELLS**
  - **MACROPHAGES**
  - **B CELLS**
- **ACCESSORY CELLS**

CELLS OF ADAPTIVE IMMUNITY

- The cells of the adaptive immune system interact with the environmental agent in a highly discriminative way, i.e., they display specificity, heterogeneity, and memory.

- These functions are primarily carried out by two types of cells that are involved in the recognition of antigen:
  - the thymus-dependent or T lymphocytes, which participate in cellular responses against intracellular pathogens, organ transplants, and malignant cells
  - the bone marrow or bursal-dependent B lymphocytes, which provide humoral immunity, i.e., antibody-mediated immunity against extracellular pathogens, their toxins, and other environmental substances.

- A third group of cells are those which are involved in the presentation of antigen to T cells, i.e., APCs, include dendritic cells, macrophages, and B cells.

- APCs take up predominantly protein antigens, cut them into peptides, bind the peptides to MHC molecules, and display them on their cell surface, where they can be recognized and bound by antigen receptors on T lymphocytes.

Antigen Presenting Cells

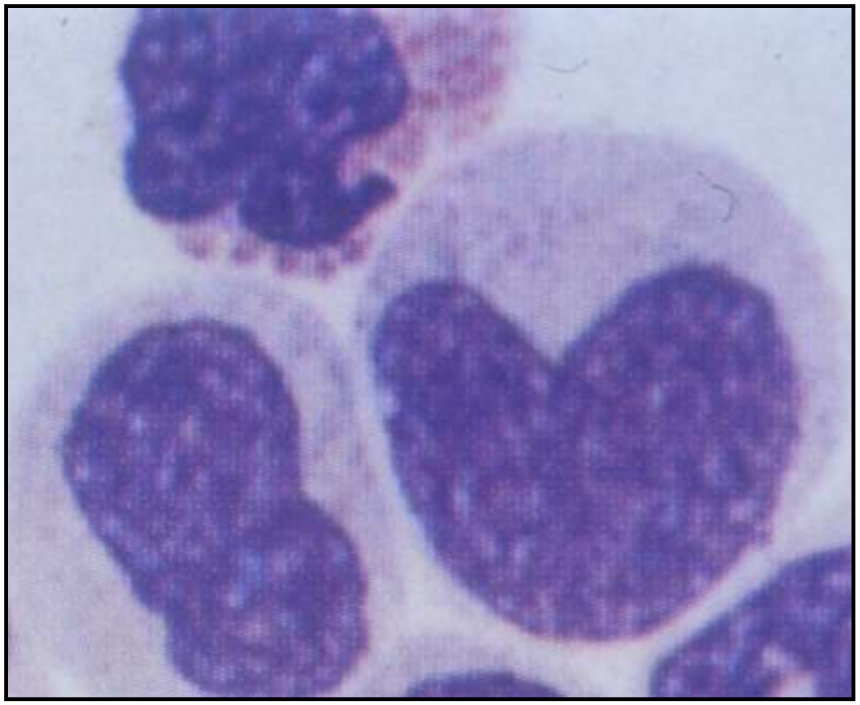
- T cell–dependent antigens' acquired immune responses typically require antigen-presenting cells (APCs) to present antigen-derived peptides within major histocompatibility complex (MHC) molecules.

- **Intracellular antigens** (e.g., viruses) can be processed and presented to CD8 cytotoxic T cells by any nucleated cell expressing class-I MHC molecules.

- **Extracellular antigens** (e.g., from many bacteria) must be processed into peptides and complexed with surface class II MHC molecules on professional APCs to be recognized by $CD4^+$ helper T (Th) cells. The following cells constitutively express class-II MHC molecules and therefore act as professional APCs:
    - Macrophages
    - Dendritic cells
    - B cells
    - Other cells (FDCs, Thymic cells)

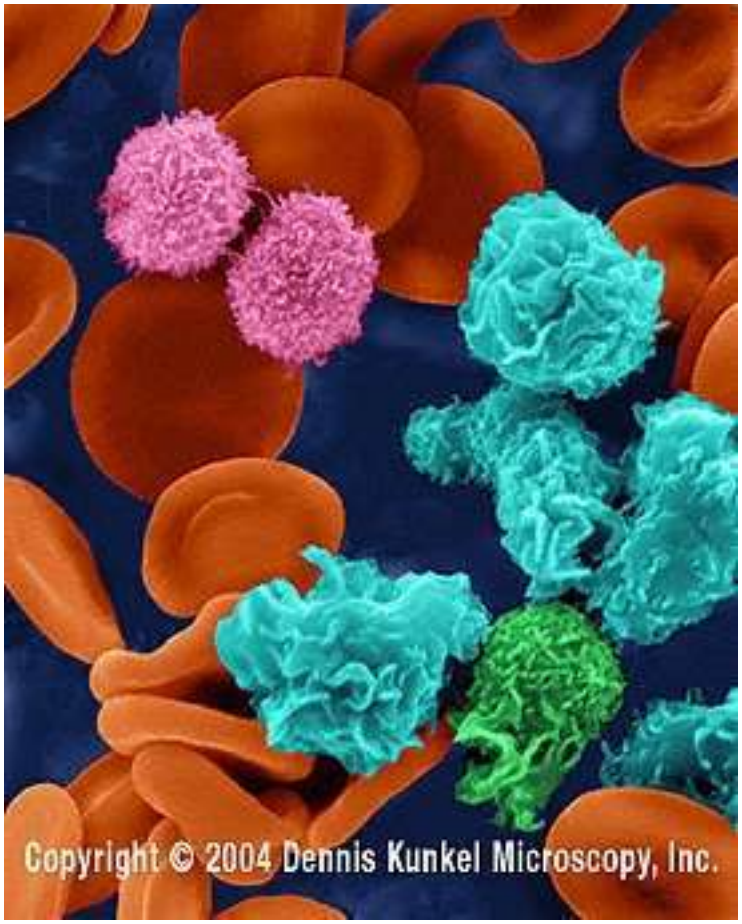
MACROPHAGES

Macrophages



- CD14 membrane marker.
- phagocytosis, antigen presentation, **“call for help”** by secreting cytokines, tissue repair
- monocytes in blood, cells of RES in tissues
- characteristic nucleus

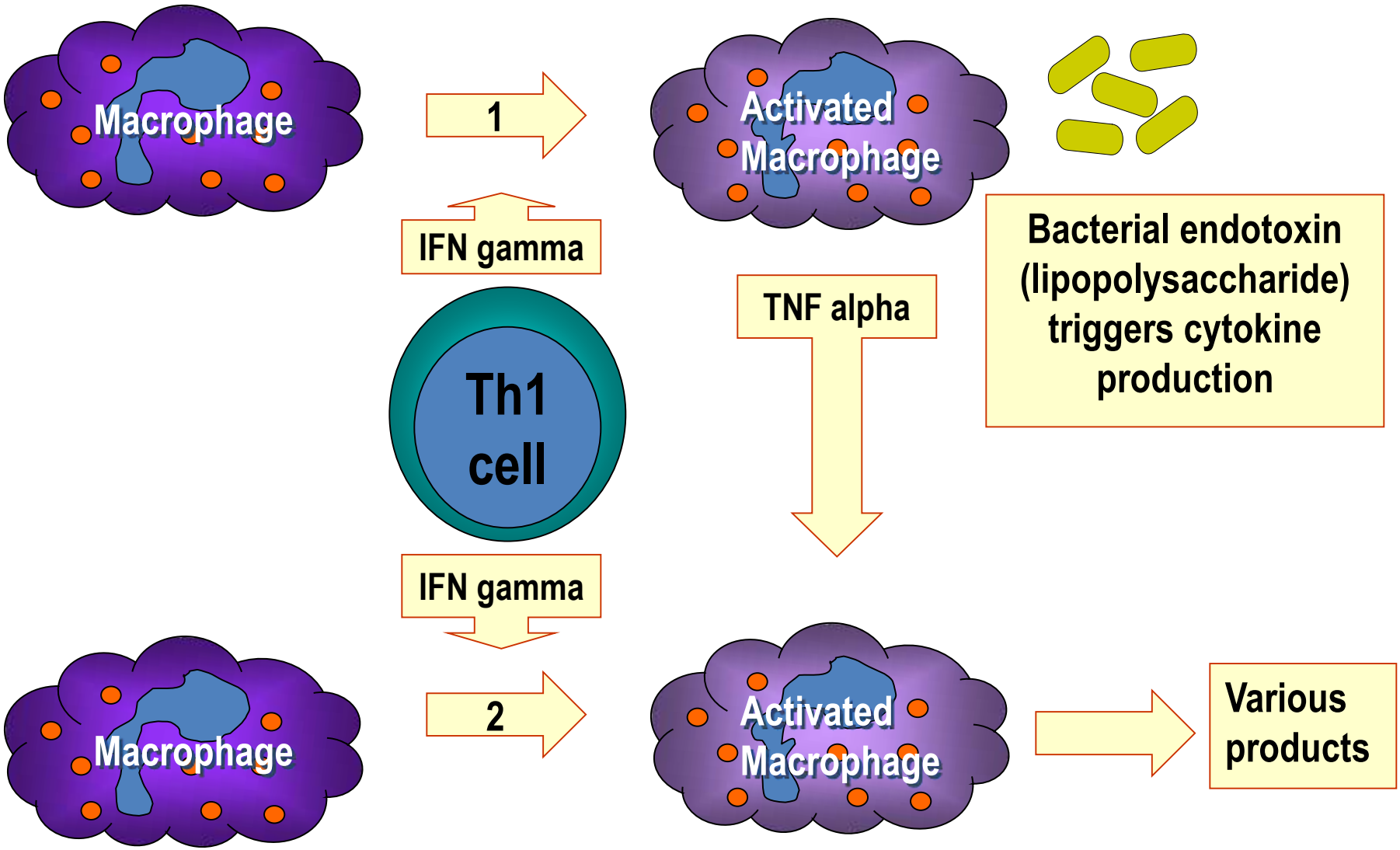
Development of monocyte into macrophage



Monocyte – green
Macrophage - blue

- Monocytes develop into about 8 h into macrophages under the influence of macrophage colony-stimulating factor (M-CSF), secreted by various cell types (e.g, endothelial cells, fibroblasts).
- At infection sites, activated T cells secrete cytokines (e.g. , IFN-γ) that induce production of macrophage migration inhibitory factor, preventing macrophages from leaving.

Mechanism of Macrophage Activation



Two major mechanisms activate macrophages:

- **IFN-γ produced by NK or Th1 cells plus bacterial endotoxin (LPS)**
- **IFN-γ produced by Th or Th1 cells plus TNF-α**

Resting Macrophages

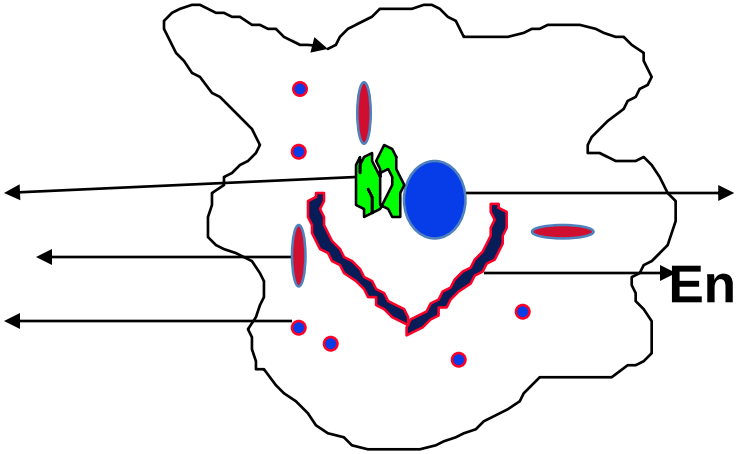
Golgi Apparatus

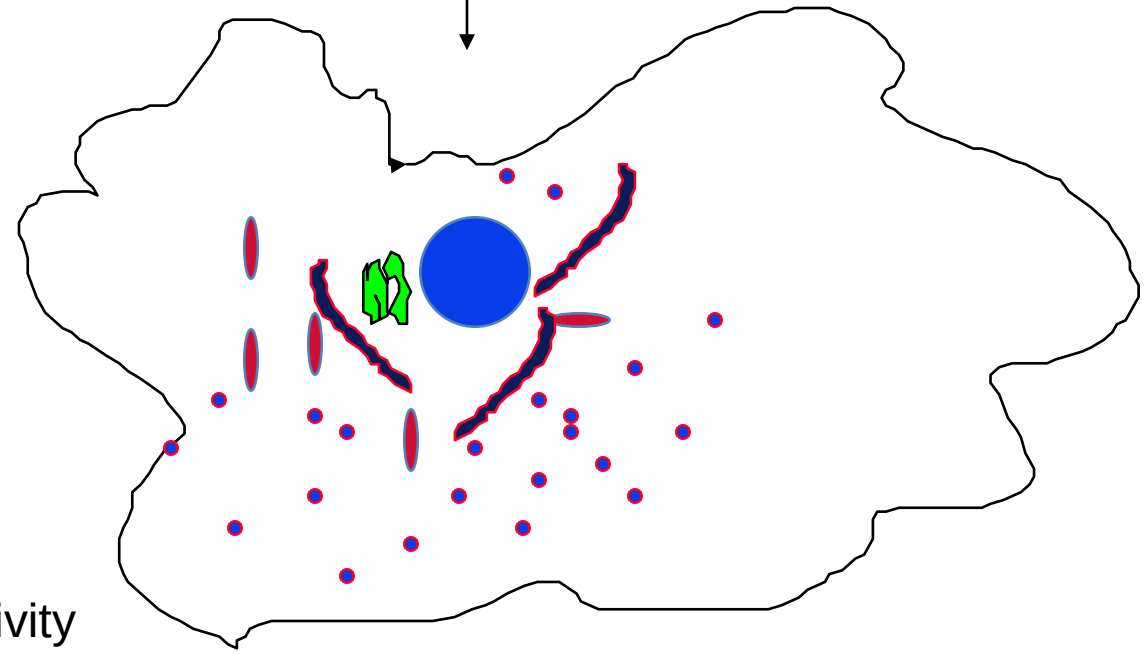
Mitochondria

Lysosomes

Nucleus

Endoplasmic Reticulum(ER

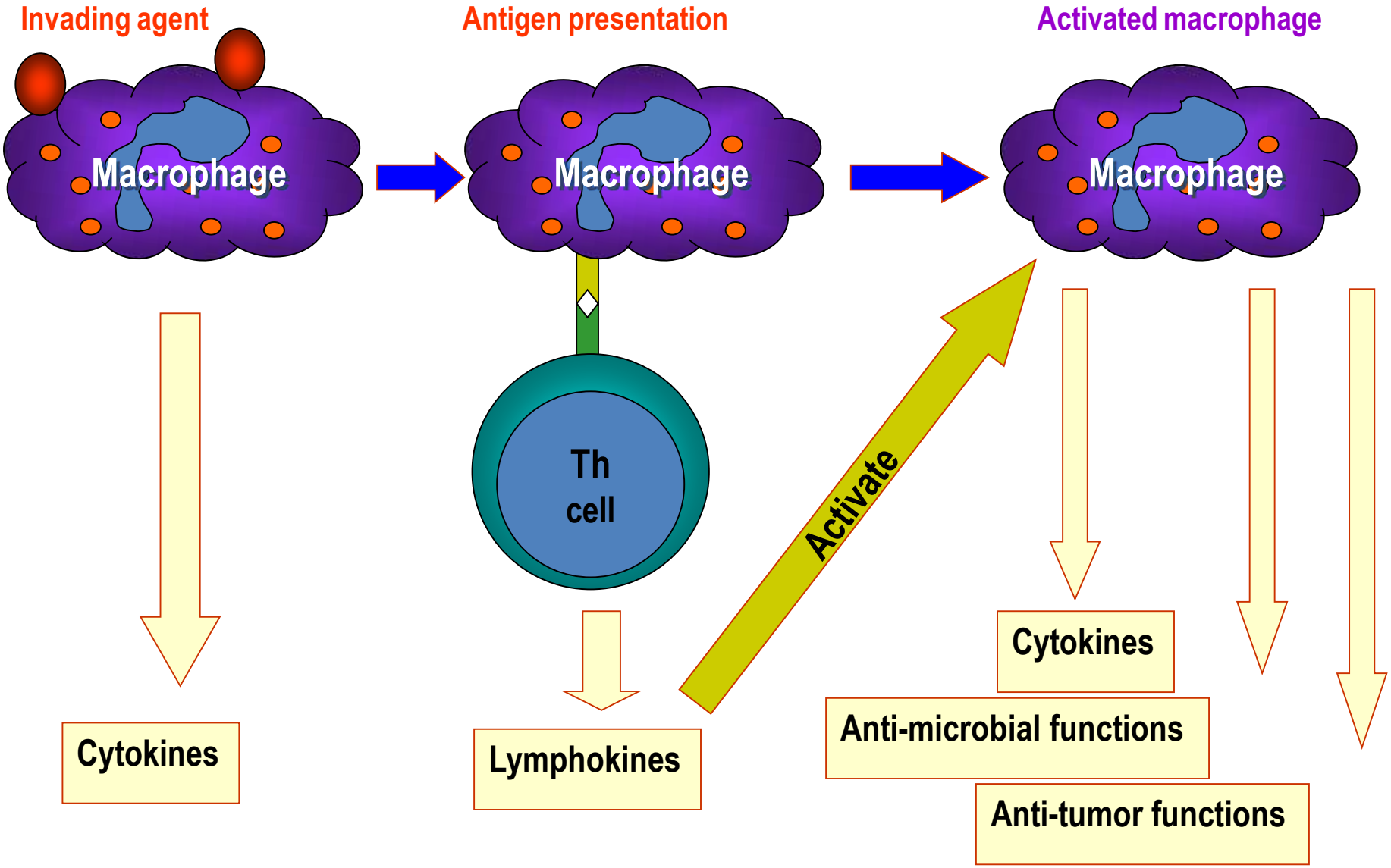




Activated Macrophage
or
Angry Macrophage

- Increased size
- Increased motility
- Increased ER
- Increased lysosomal activity

Central Role of Macrophages in Innate and Adaptive Immunity



Detailed Functions of Macrophages

Inflammation – Fever, Production of: IL-6, TNF-alpha, IL-1 – act as pyrogen

Immunity
Selection of lymphocytes to be activated:
IL-12 results in Th1 activation
IL-4 results in Th2 activation
Activation of lymphocytes:
Production of IL-1
Processing and presentation of antigen

Reorganization of tissues,
Secretion of a variety of factors:
Degradative enzymes (elastase, hyaluronidase, collagenase)
Fibroblast stimulation factors
Stimulation of angiogenesis

Damage to tissues
Hydrolases, Hydrogen peroxide production
Complement C3a
TNF alpha production

Antimicrobial action (Phagocytosis)
O2–dependent production of:
hydrogen peroxide, superoxide, hydroxyl radical, hypochlorous acid
O2-independent production of:
acid hydrolases, cationic proteins, lysozyme

Anti-tumor activity produced by:
Toxic factors
Hydrogen peroxide
Complement C3a
Proteases, Arginase
Nitric oxide
TNF alpha

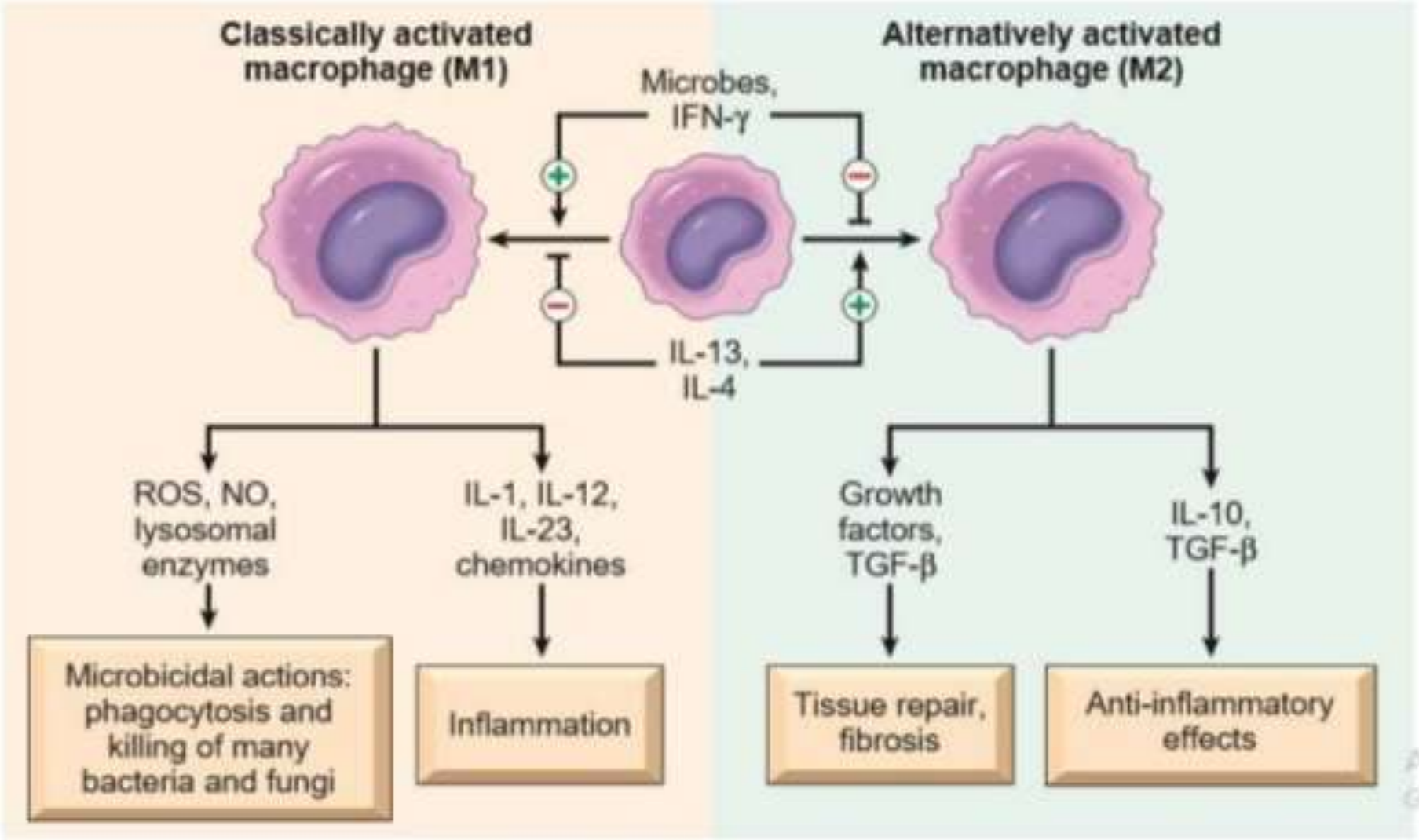
Types of Macrophages

Macrophage Subtypes

Characteristics	M1	M2
Activation agent	Stimulation of Toll-like receptors IFN-gamma (a cytokine produced by Th1 cells)	IL-4 and IL-13 (cytokines produced by Th2 cells)
Cytokines produced	Proinflammatory cytokines (eg, TNF-alpha)	Immunosuppressive cytokines (eg, IL-10)
Other functions	Promote Th1 responses Are strongly microbicidal	Promote tissue remodelling

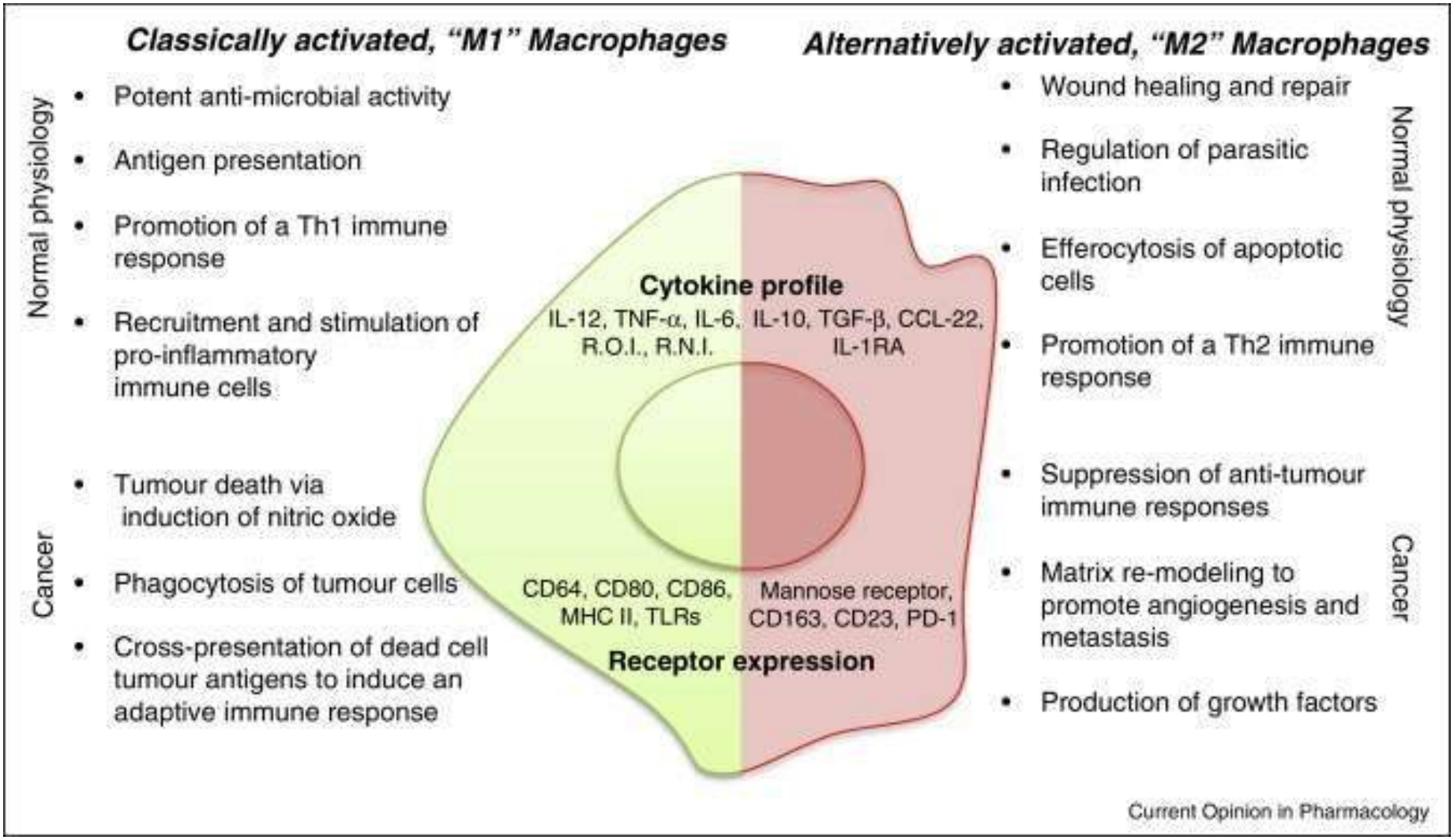
IFN = interferon; IL = interleukin; Th1 cells = type 1 helper T cells; Th2 cells = type 2 helper T cells; TNF = tumor necrosis factor.

Activation pathways

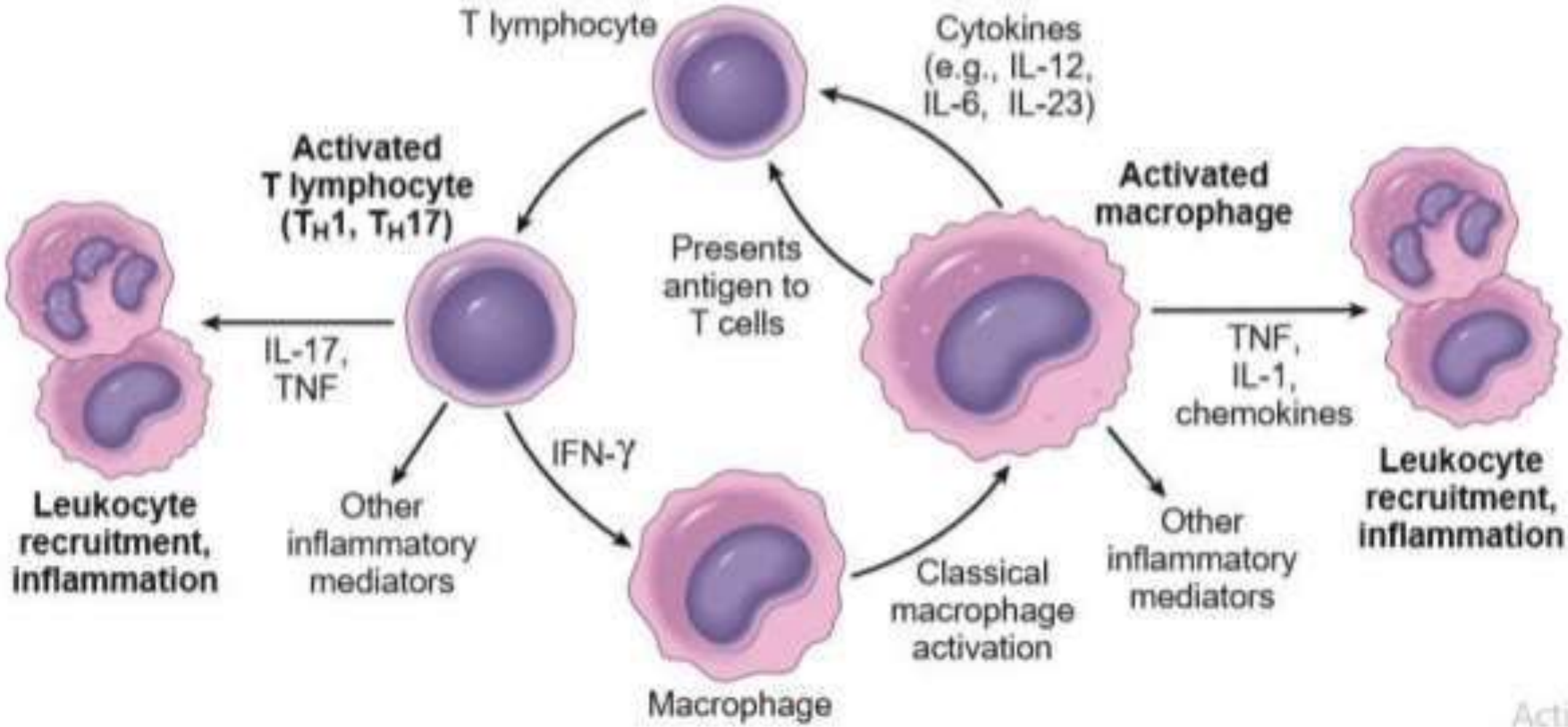


Differences between M1 and M2 Macrophages

	M1 Macrophages	M2 Macrophages
Activation	Classical (Th1)	Alternative (Th2)
Stimuli	IFN-γ, LPS	IL-4, IL-13, Immune Complexes, LPS, Glucocorticoids, Etc.
Proinflammatory Cytokines	High Levels	Low Levels
Antigen Presentation	Yes	No
Nitric Oxide Production	Yes	No
Function	Kill Microbes	Build Extracellular Matrix


Classically activated, "M1" Macrophages
Alternatively activated, "M2" Macrophages
Normal physiology
Potent anti-microbial activity
Antigen presentation
Promotion of a Th1 immune response
Recruitment and stimulation of pro-inflammatory immune cells
Cancer
Tumour death via induction of nitric oxide
Phagocytosis of tumour cells
Cross-presentation of dead cell tumour antigens to induce an adaptive immune response
Cytokine profile
IL-12, TNF-α, IL-6, R.O.I., R.N.I.
IL-10, TGF-β, CCL-22, IL-1RA
CD64, CD80, CD86, MHC II, TLRs
Mannose receptor, CD163, CD23, PD-1
Receptor expression
Normal physiology
Wound healing and repair
Regulation of parasitic infection
Efferocytosis of apoptotic cells
Promotion of a Th2 immune response
Cancer
Suppression of anti-tumour immune responses
Matrix re-modeling to promote angiogenesis and metastasis
Production of growth factors
Current Opinion in Pharmacology

Macrophage-Lymphocyte interaction



DENDRITIC CELLS


DENDRITIC CELL

DENDRITIC CELLS

- discovered by Ralph Steinman and Zanvil Cohn in 1973
- have numerous cytoplasmic processes giving ‘tree-like’ or dendritic shapes, hence so called
- function as the ‘**sentinels’** of the immune system
- present in all tissues except brain, eye and testes
- activated directly by PAMPs and indirectly by inflammatory cytokines
- induce pro-inflammatory cytokines and activate innate lymphocytes
- **DCs exist in 2 functionally different states (mature and immature):** immature form phagocytose antigen and then becomes mature, stop phagocytosing, and goes to the lymph node to deliver the antigen to T cells**.**

(immature and mature DC’s function differently)

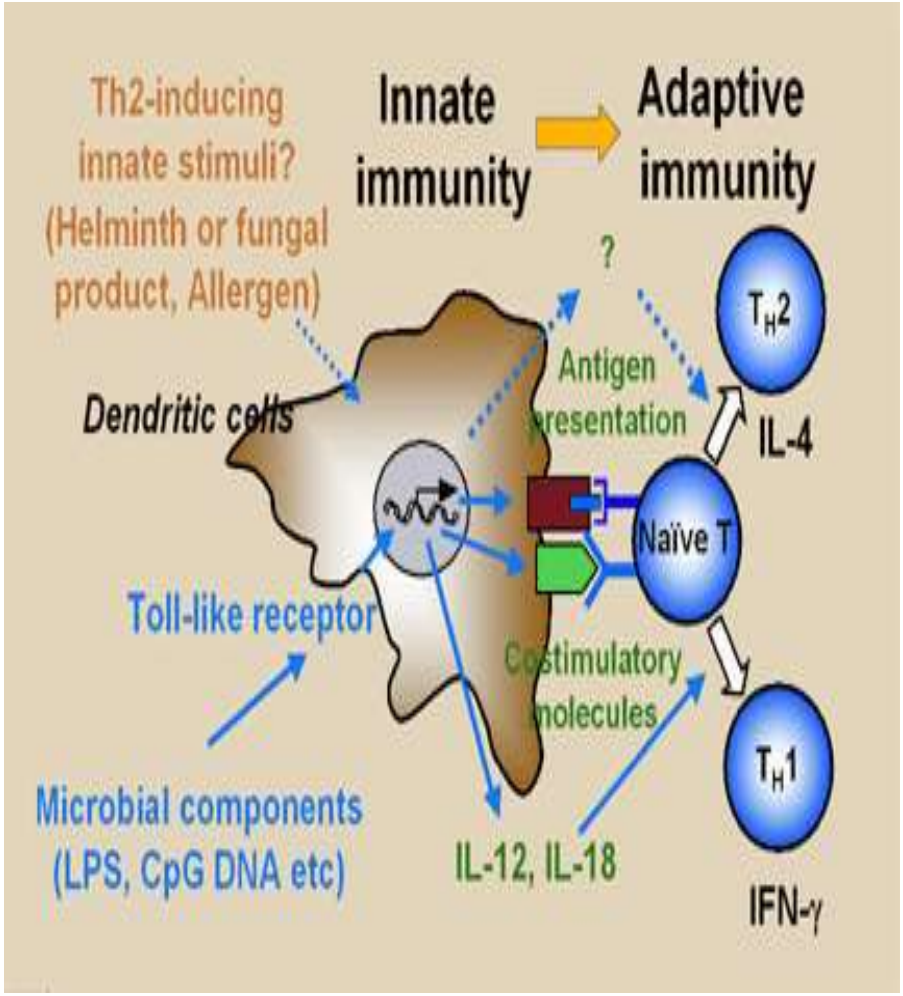
- if no interaction with an antigen, immature DCs die with in 2-4 days.

DENDRITIC CELLS (contd.)

- DCs have been referred to as “professional” APCs, since the principal function of DCs is to present antigens.

  (DCs initiate immune responses that are conducted by other cell types)
- DCs are >100 times more powerful than macrophages or B cells in presenting antigens
- Only DCs have the ability to induce a primary immune response in resting naïve T lymphocytes.
- DCs are capable of capturing antigens, processing them, and presenting them on the cell surface along with appropriate co-stimulatory molecules to T cells.
- DC are a very plastic cell population that can shape its phenotype to the microenvironment and to homeostatic state of the tissue where it is located

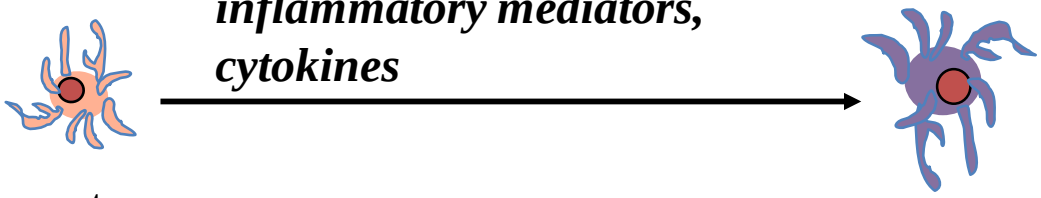
DCs - the link between the innate and adaptive immune response



- as immature cells, they act as sensors of the environment,
- recognize PAMPs via TLRs and undergo activation
- produce cytokines which participate in innate immunity
- undergo maturation and migration to secondary lymphoid organs where they stimulate naïve T cells to induce adaptive immunity

Immature and mature DCs function differently

Contact with antigen activates immature DC leading to maturation, e.g. bacterial products, inflammatory mediators, cytokines



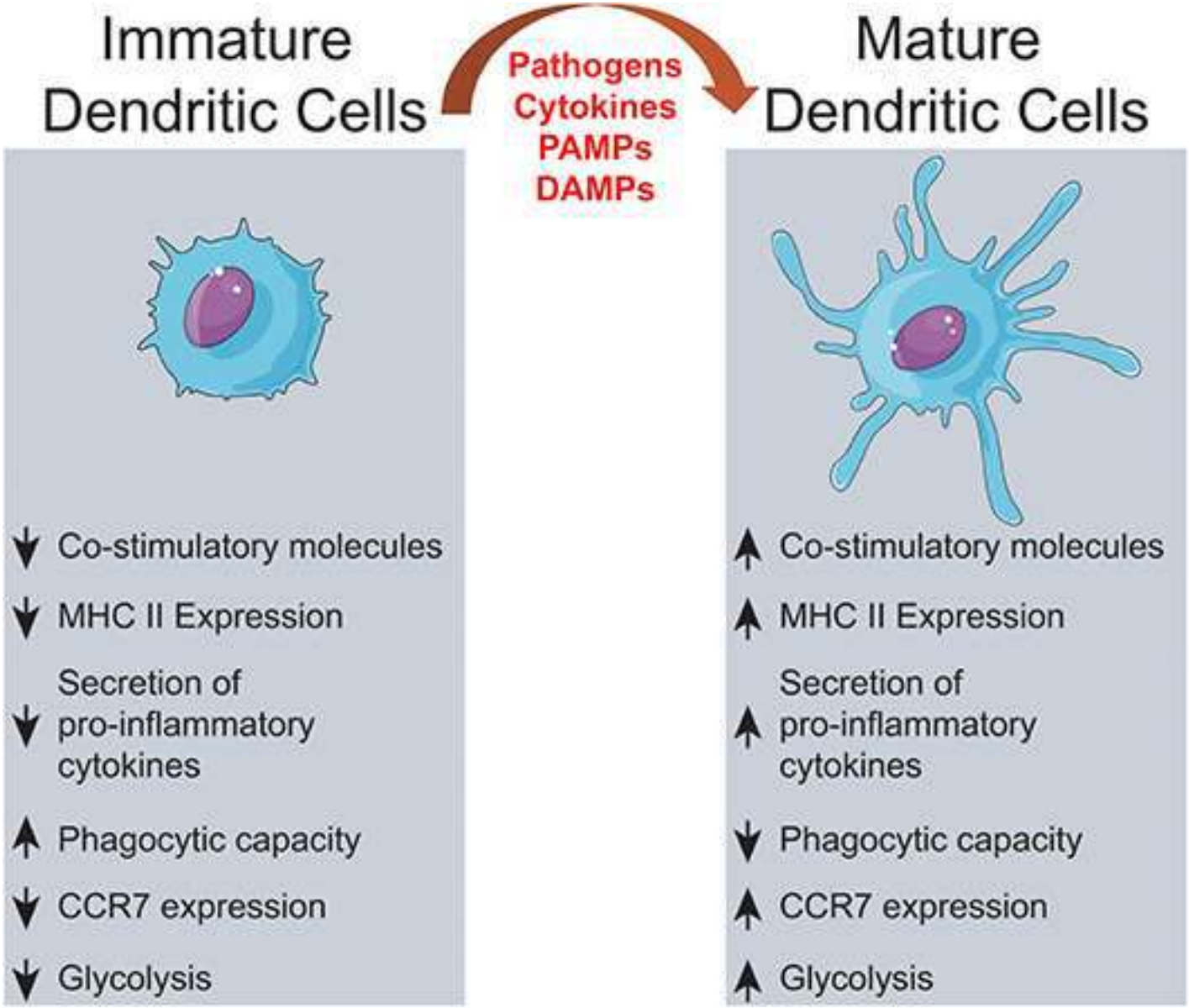
Immature
Dendritic Cells

Excellent at capturing antigen

Mature
Dendritic Cells

Excellent at Processing antigen

Differences between Immature and Mature DCs

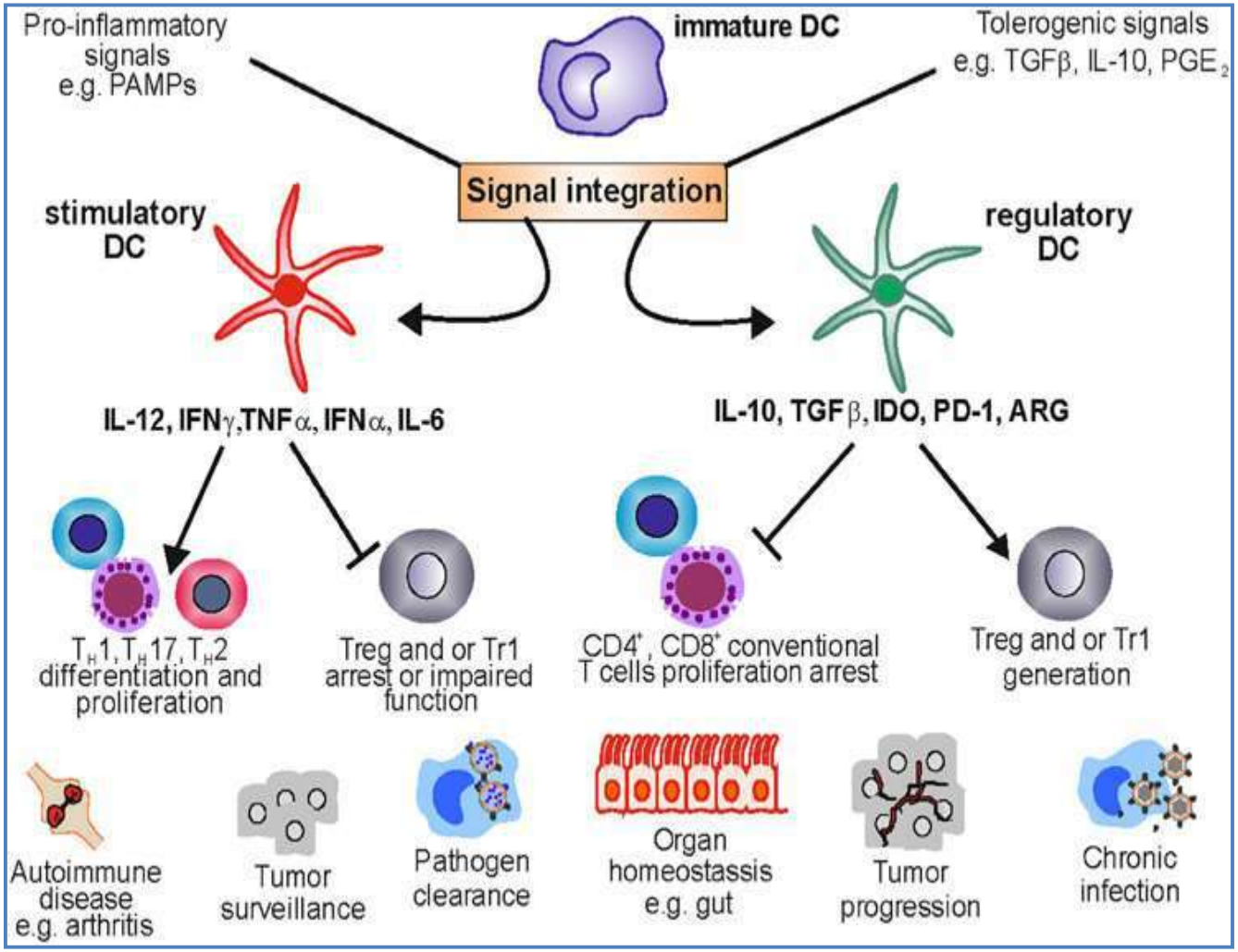


PRINCIPAL FUNCTIONS OF DENDRITIC CELLS

The functions of DCs falls broadly into three categories:

- DCs function is antigen processing and presentation and activation of T cells (myeloid DCs)
- DCs appear to work to maintain immune memory in tandem with B cells (plasmacytoid DCs)
- DCs function for inducing and maintaining immune tolerance/regulation (myeloid DCs)

Thus we have immuno - stimulatory and regulatory DCs



Under **pro-inflammatory conditions** stimulatory DCs **promote an immune response** by stimulating T cell proliferation

Under a **tolerogenic environment** regulatory DCs **control immune** by suppressing T cell activation and proliferation

Types of Dendritic Cells

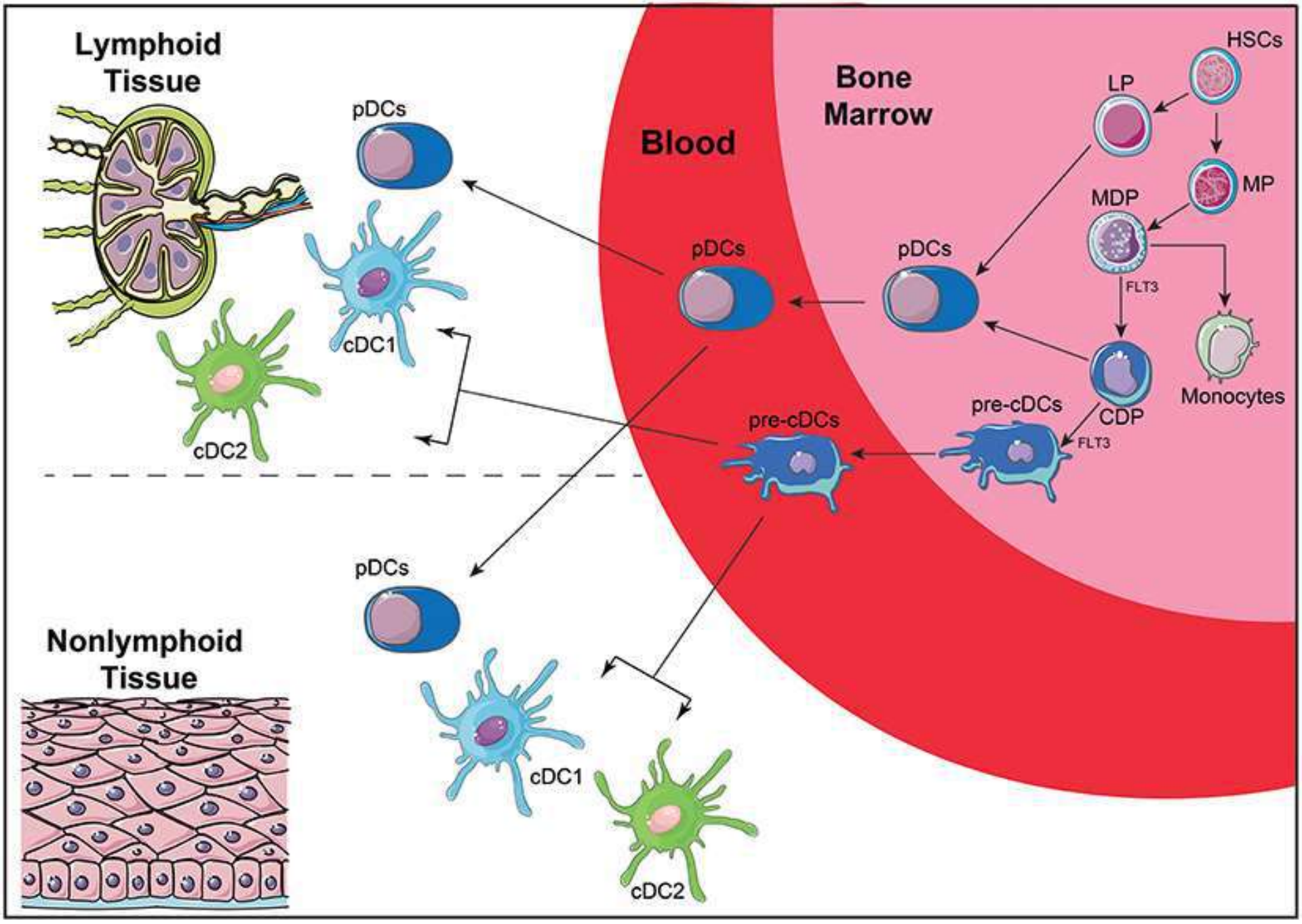
DCs are classified on the basis of progenitors, transcription factors, cytokines produced, surface markers, functions, and anatomical location into the following types:

- **Classical DCs**
  - **Plasmacytoid DCs (pDC)**
  - **Conventional or Myeloid DCs (cDC)**
    - myeloid/conventional DC1 (cDC1)
    - myeloid/conventional DC2 (cDC2)
- **Monocyte-derived DC (mo-DC)**
- **Langerhans cells (LC)**

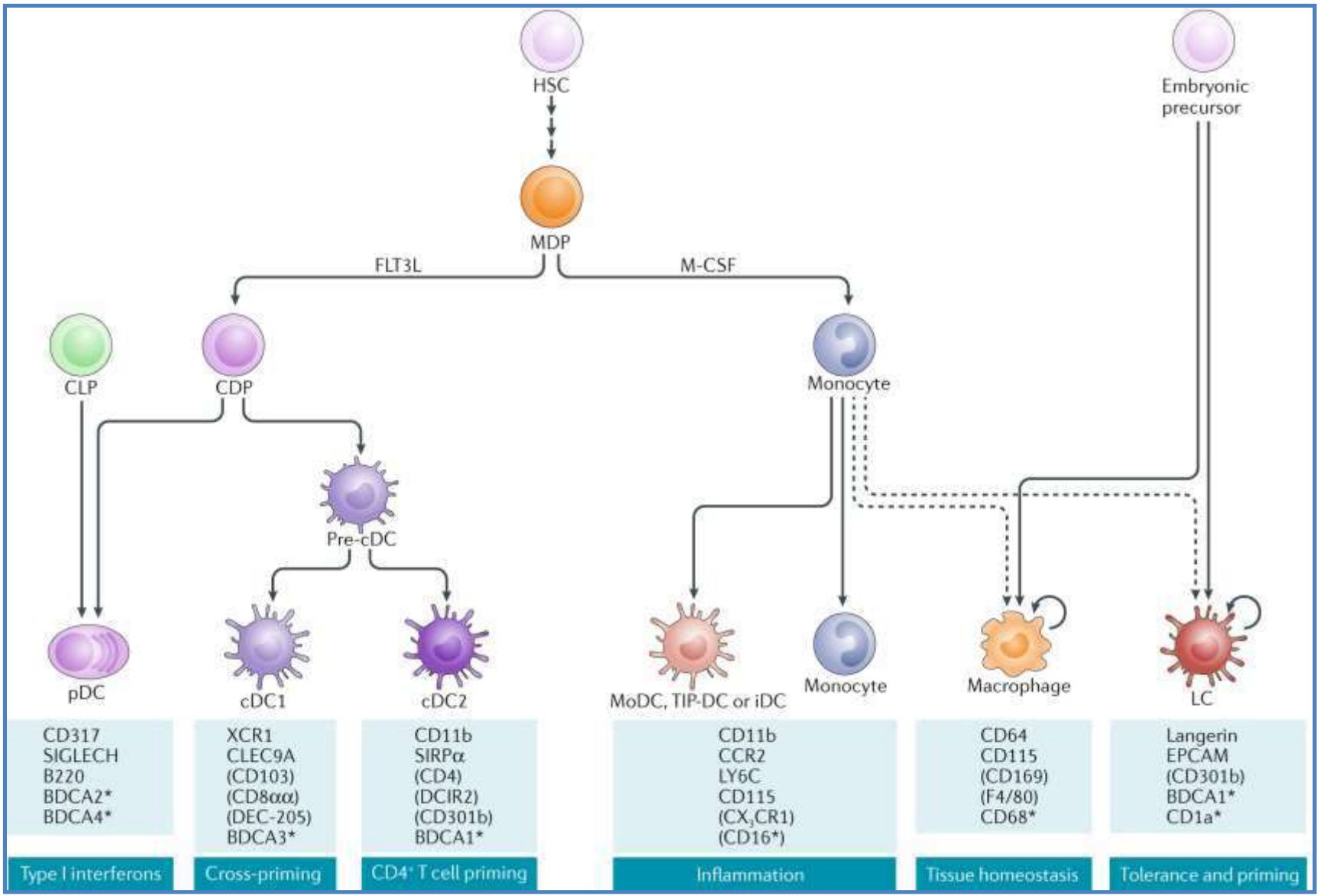
(The existence of several different types of DC, each specialized to respond to particular pathogens and to interact with specific subsets of T cells, expands flexibility of immune system to react appropriately to a different pathogens and danger signals)

Classical DCs – types and location

- Three major DC subsets: plasmacytoid DC (pDC), myeloid/ conventional DC1 (cDC1), and myeloid/conventional DC2 (cDC2).
- These DC subsets are a product of the core lympho-myeloid pathway rather than 'dual' lymphoid and myeloid origin as thought earlier.
- Each DC subset develops under the control of a specific repertoire of transcription factors, such as interferon regulatory factors 8 and 4 (IRF8 and IRF4) .
- pDCs are located in blood and lymhpoid tissues, but are also found in inflamed or transformed non-lymphoid tissues
- cDC1 are found in blood and among resident DC of lymph node, tonsil, spleen and bone marrow and non-lymphoid tissues, skin, lung, intestine and liver.
- cDC2 is the major population of myeloid cDC in human blood, tissues and lymphoid organs


Lymphoid Tissue
pDCs
cDC1
cDC2
Blood
Bone Marrow
HSCs
LP
MP
MDP
FLT3
pDCs
pDCs
pDCs
pre-cDCs
pre-cDCs
CDP
FLT3
Monocytes
Nonlymphoid Tissue
pDCs
cDC1
cDC2

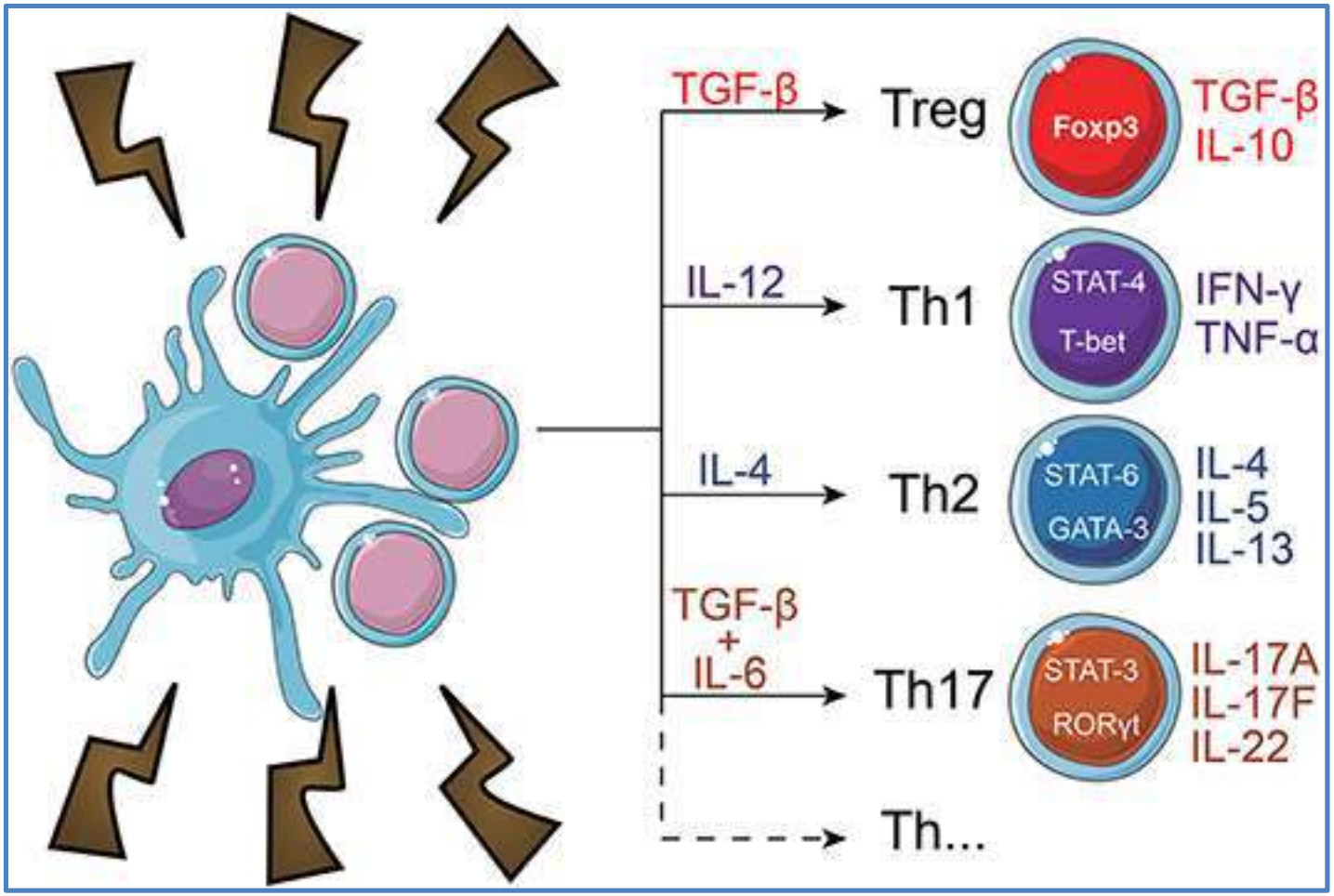
Detailed Functions of Different Types Dendritic cells



Classical DCs - functions

- Three types of signals are delivered by DCs to T cells and instruct their functional polarization:
  - **presentation of antigenic peptides in association with MHC molecules,**
  - **co-stimulation, and**
  - **release of cytokines**.
- Different **cytokines induce distinct types of helper T-cell responses**. For example, IL-12 primarily promotes Th1 and IL-4 promotes Th2.
- Co-stimulation and cytokine signals can be either **activating** (e.g., CD86 and IL-12) or **inhibitory** (e.g.,PD-1L IL-10).
- Different DC subsets can exert similar or complementary functions depending on the physiopathological context.

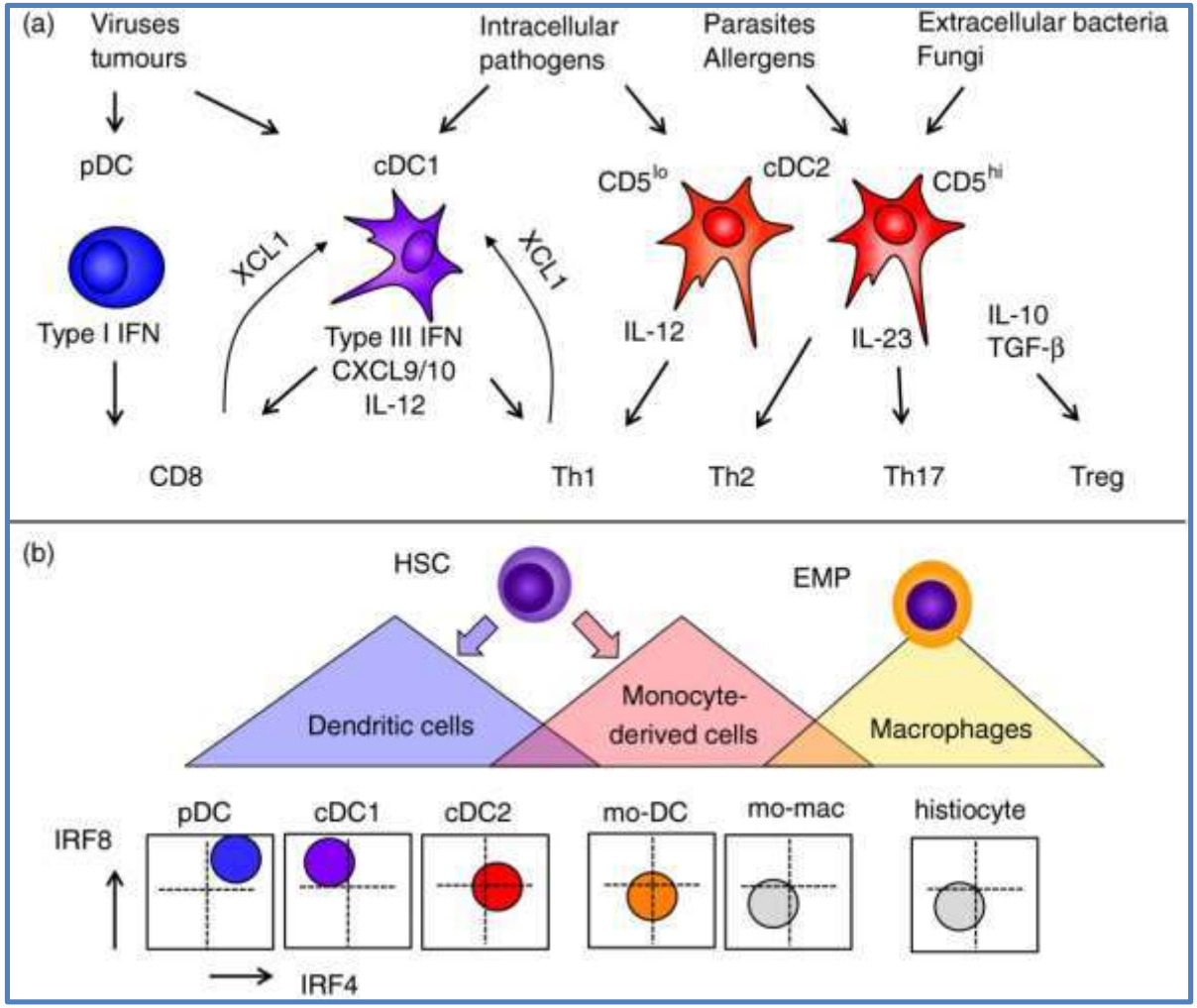
Classical DCs



Classical DCs – functions (contd.)

- **pDCs** sense ssRNA and dsDNA (via TLR7 and TLR9) and produces type I and type III interferons and secretion of cytokines. They are **mainly involved in innate defences against viruses and tumors**. They also activate Th1 response and promote $CD8^+$ T cells
- **cDC1** recognize viral and intracellular antigens (via TLR3, TLR9 and TLR19) and produces IL-12 and type III interferon. They have capacity to cross-present exogenous antigens via MHC class I to **activate $CD8^+$ T cells and to promote Th1 and NK cells responses against intracellular pathogens and tumors (CMI)**
- **cDC2** responds to LPS, flagellin, poly IC, etc (via lectins, TLRs, NORs and RIG-I-like receptors) and secrete IL-12, IL-23, IL-1, TNF-*α*, IL-8, IL-10 and TGF-β. They are potent in the **priming of CD4+ cells (Th1, Th2, Th17) and immunity (AMI and CMI) against extracellular pathogens (bacteria, parsites, fungi).**
- **cDC2 cells** can also cross-present antigens through IL-12. The **activation of Treg cells** through IL-10 and TGF-β **promotes regulatory functions**

Classical dendritic cell subsets: an update



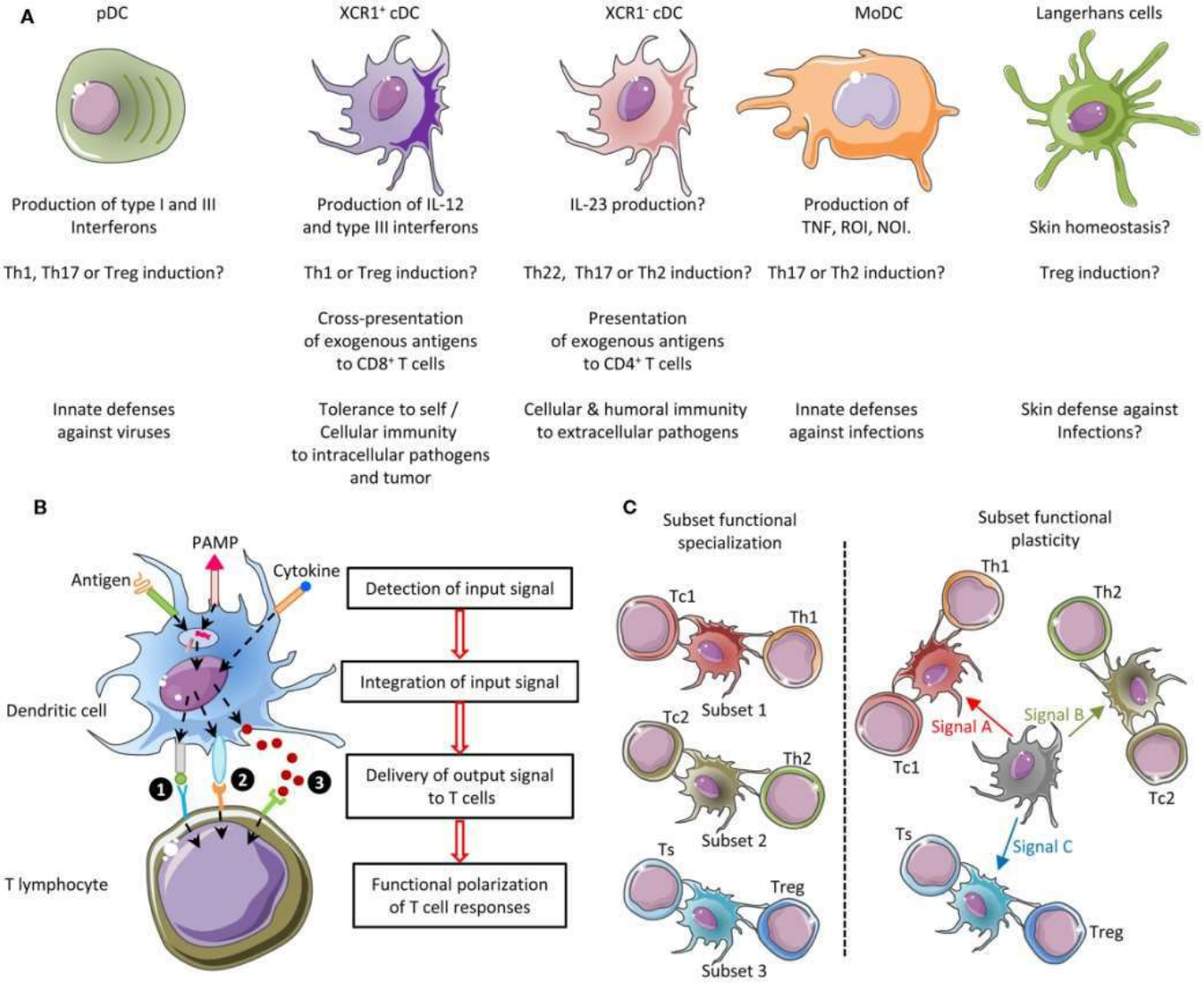
Immunology, Volume: 154, Issue: 1, Pages: 3-20, First published: 03 January 2018, DOI: (10.1111/imm.12888)

Monocyte-derived inflammatory DC (mo-DC)

- **mo-DCs are distinct entities from cDC and pDC being derived from primitive myeloid progenitors arising in the yolk sac**
- Populations of monocyte-derived cells exist in steady-state tissues, including the skin, lung and intestine
- During inflammation, monocyte-derived cells expand resident populations many-fold, hence also termed as **'inflammatory dendritic cell'** .
- myeloid cDC2 share the most markers with monocytes and can be difficult to dissociate entirely from mo-DC.
- mo-DC secrete IL-1, TNF-*α*, ROI, NOI, IL-12 and IL-23
- mo-DCs are **cells of inflammatory response and are involved in innate defences** through ROIs and NOIs
- Stimulate **Th2 and Th17 (?) cells (AMI)**
- Surprisingly mo-DCs have been found to **induce regulatory T cells in cancer** patients
- mo-DC are able to **present antigens in the context of both MHC class I and class II molecules** and, hence, can be used to generate therapeutic cancer vaccines.

Langerhans cells (LC)

- **Langerhans cells are specialized DC that inhabit the basal epidermis and other stratified squamous epithelia.**
- LCs cells are phylogenetically ancient and share a primitive origin with tissue macrophages
- Once generated, LCs are **capable of local self-renewal**, independently of the bone marrow,
- They express the C-type lectin langerin and the MHC class I molecule .
- **LC capture antigen, mature and migrate to skin-draining lymph nodes, where they appear in the T-cell areas**
- When the skin becomes inflamed, local production of TNF-α and IL-1β stimulate LC to lose their connections with the surrounding epithelium and migrate across the basement membrane into the afferent lymphatics.
- LCs are **potent cross-presenting DC** with high IL-15 production and the ability to **present antigens and stimulate CD8 T cells**
- LCs play an important role in **maintaining epidermal health and tolerance to commensals**, while retaining the ability to respond to selected intracellular pathogens and viruses under inflammatory conditions


A
pDC
XCR1+ cDC
XCR1- cDC
MoDC
Langerhans cells
Production of type I and III Interferons
Production of IL-12 and type III interferons
IL-23 production?
Production of TNF, ROI, NOI.
Skin homeostasis?
Th1, Th17 or Treg induction?
Th1 or Treg induction?
Th22, Th17 or Th2 induction?
Th17 or Th2 induction?
Treg induction?
Cross-presentation of exogenous antigens to CD8+ T cells
Presentation of exogenous antigens to CD4+ T cells
Innate defenses against viruses
Tolerance to self / Cellular immunity to intracellular pathogens and tumor
Cellular & humoral immunity to extracellular pathogens
Innate defenses against infections
Skin defense against Infections?
B
PAMP
Antigen
Cytokine
Dendritic cell
T lymphocyte
Detection of input signal
Integration of input signal
Delivery of output signal to T cells
Functional polarization of T cell responses
C
Subset functional specialization
Tc1
Th1
Subset 1
Tc2
Th2
Subset 2
Ts
Treg
Subset 3
Subset functional plasticity
Th1
Th2
Signal A
Signal B
Tc1
Tc2
Signal C
Ts
Treg

Type of DC	Lineage	Location	Principal cytokines	Activation of	Functions
pDC (plasmacytoid)	lympho-myeloid	blood, lymhpoid tissues, inflamed or transformed non-lymphoid tissues	IFN-α and γ	Th1, Th17, and $CD8^+$ T cells	Innate defences against viruses and tumors; promote CMI
cDC1 (myeloid)	-do-	blood, resident DC of lymphoid tissues, non-lymphoid tissues, skin, lung, intestine and liver.	IL-12 and IFN-γ	Th1, $CD8^+$ T cells and NK cells	cross-present exogenous antigens ; promote CMI against intracellular pathogens and tumors
cDC2 (myeloid)	-do-	major population of myeloid cDC in blood, tissues and lymphoid organs	IL-12, IL-23, IL-1, TNF-*α*, IL-8, IL-10 and TGF-β.	Th1, Th2, Th17 and Treg cells	priming of CD4+ cells (Th1, Th2, Th17) and immunity (AMI and CMI) against extracellular pathogens (bacteria, parsites, fungi).
mo-DC (monocyte derived)	monocyte	inflamed tissues	TNF, ROI, RNI	Th2 or Th17 (?)	cells of inflammatory response, innate defences Stimulate AMI
LC (Langerhans' cells)	tissue macrophages	basal epidermis and other stratified squamous epithelia.	IL-15	Treg (?)	present antigens entering from dermis to CD8 T cells in LN; tolerance; skin health

FOLLICUALR DENDRITIC CELLS

Follicular Dendritic Cells (FDCs)

- Follicular DCs are a **non-migratory population found in primary and secondary follicles of the B-cell areas** of lymph nodes, spleen, and MALT
- **non-hematopoietic cells of mesenchymal (stromal) origin**.
- resides in the central region of primary follicles and in GC of SLOs in intimate interaction with follicular B cells.
- Follicular DCs have **high expression of complement receptors** CR1 and CR2 (CD 35 and CD 21 respectively) and **Fc-receptor** (FcγRIIb-CD32).
- **FDCs are non-phagocytic cells, do not express class II MHC molecules, and therefore, do not present antigen to Th cells**
- **Instead, FDCs have the unique capacity to bind and retain native antigen in B-cell follicles for long periods of time.**
- FDCs does not extend from the follicle to the interfollicular regions or T- cell zone. This separation from the sites of antigen processing provide a protected environment in which opsonised antigens can be displayed for a long time without being proteolyzed or removed by phagocytic cells.

Follicular Dendritic Cells (FDCs)

Caution

- “Dendritic cells" and "follicular dendritic cells" are completely different cell types that obtained similar names (dendritic = tree-like) because of their morphological appearance.
- Dendritic cells are specialized APC ingesting antigen in the periphery and presenting processed antigen on MHC II to Th cells.
- Follicular dendritic cells sit in germinal centers and use complement receptors and fix antigen-containing immune complexes on their outer surface to be presented to memory B cells

FDCs - Functions

The Cardinal Function of FDCs is Immune-Complex (IC) trapping

- FDCs are essential for production of high-affinity antibody molecules and for the development of B cell memory.

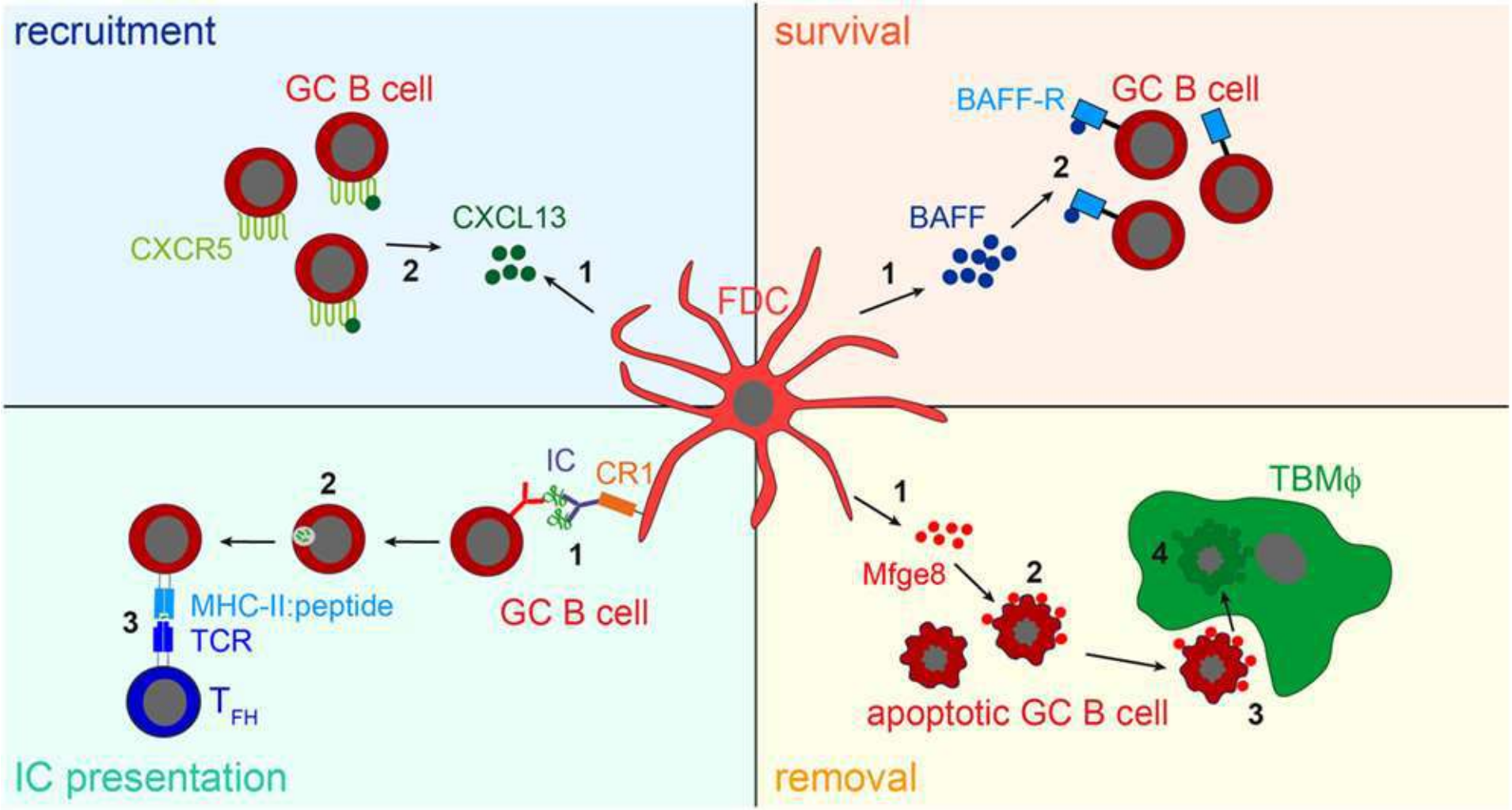
- FDCs express a variety of cell surface receptors, including Fc receptors CD23 and CD32, which bind the constant (nonvariable) region of Igs and complement receptors CD21 and CD35.

- By virtue of above receptors, FDCs have unique ability to capture and retain naive (unprocessed) antigen, via complement receptors and Fc-receptor for IgG, in the form of antigen depots called as **immune complexes (ICs).** These antigen deposits are important in the development of an already initiated primary immune response

- These ICs are then presented to germinal centre's memory B-cells, of which only B cells with high affinity B cell receptors (BCR) can bind, to start the secondary immune response.

- FDCs are main producers of the chemokine CXCL13 which attracts and organises B lymphoid cells in their respective zones in SLO

FDC influence on B cells



The Role of FDC-Bound ICs in B-Cell Responses

- **The assembly of an antigen–antibody–C3 complex on FDCs is crucial for B-cell memory**

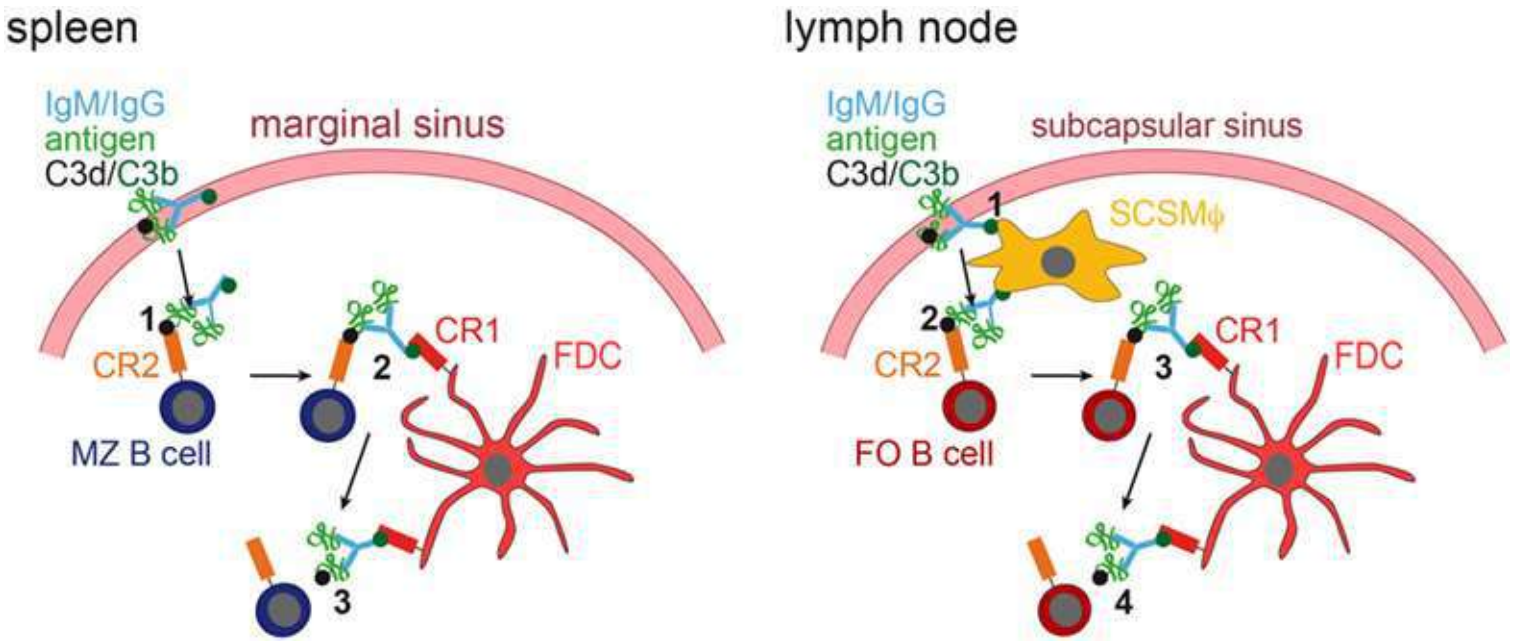
- Marginal zone (MZ) B cells in spleen and sub-capsular sinus (SCS) macrophages in lymph nodes (called as antigen transport cells-ATCS) deliver antigen as IC to FDCs in a complement receptor-dependent manner. These cells transport the antigen inside the follicle and deposit it onto FDCs.

- FDCs complement receptors 1 and 2 (CR1, CR2) and IgG receptor (FcγRIIb) are responsible for capturing of C3-containing IC.

- FDCs retain native antigen sufficiently long, protect from degradation and at the same time concentrate and polarize it at the location where many B cells reside. This strongly increases the likelihood of antigen-encounter by the rare cognate B cell.

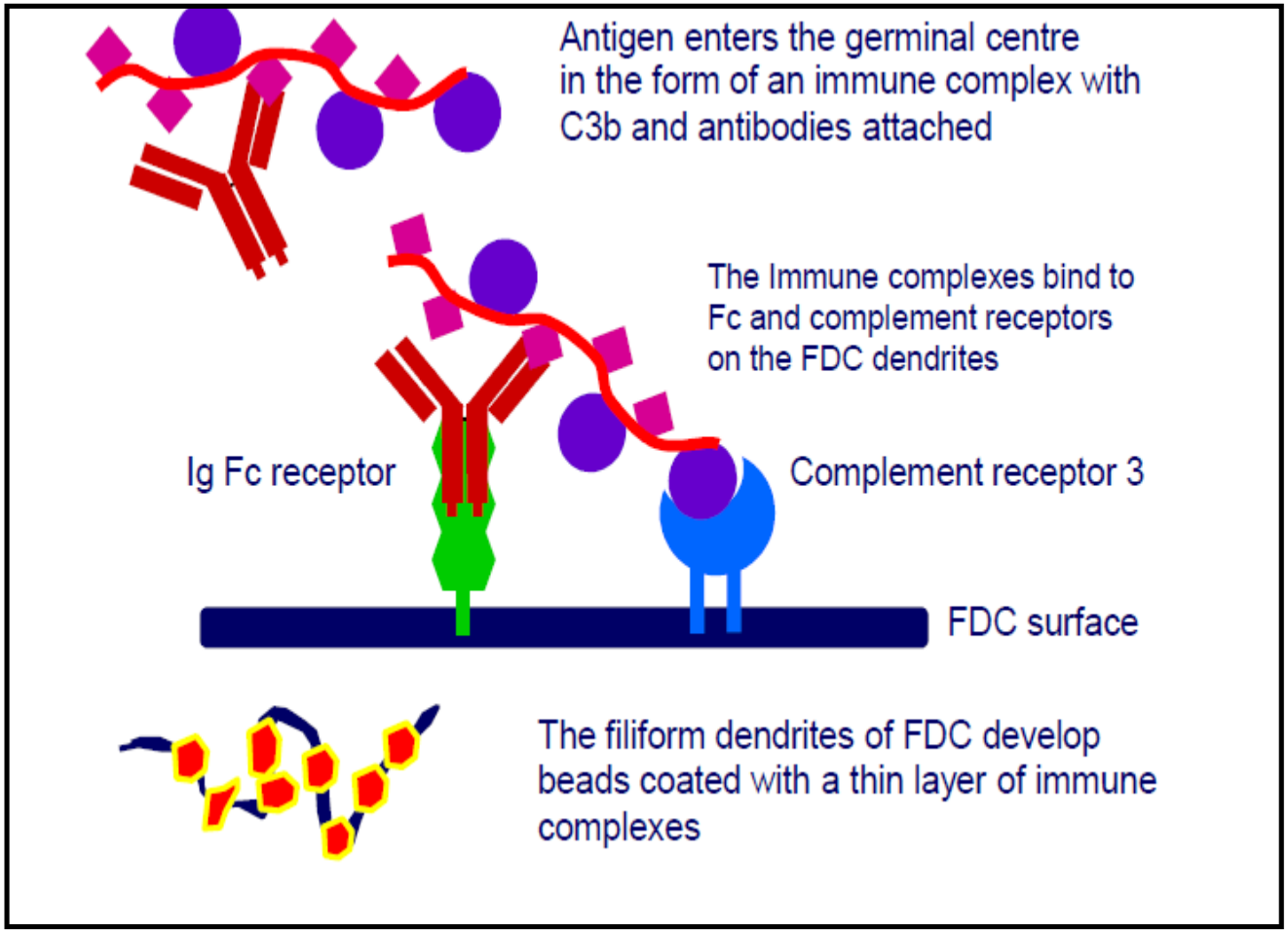
- During the GC reaction, high-affinity B cells access antigen, internalize, process, and display it to T helper cells, thereby receiving BCR stimulation as well as additional T helper cell-derived survival signals to **produce high-affinity antibody molecules and for the development of secondary immune response.**

- It is postulated that efficient mechanisms also exist, which allow FDCs to capture, retain, and present antigen in non-immunised hosts in an antibody-independent manner and, thus, can play **an important role in the initial activation of B cells, ie development of primary immune response**

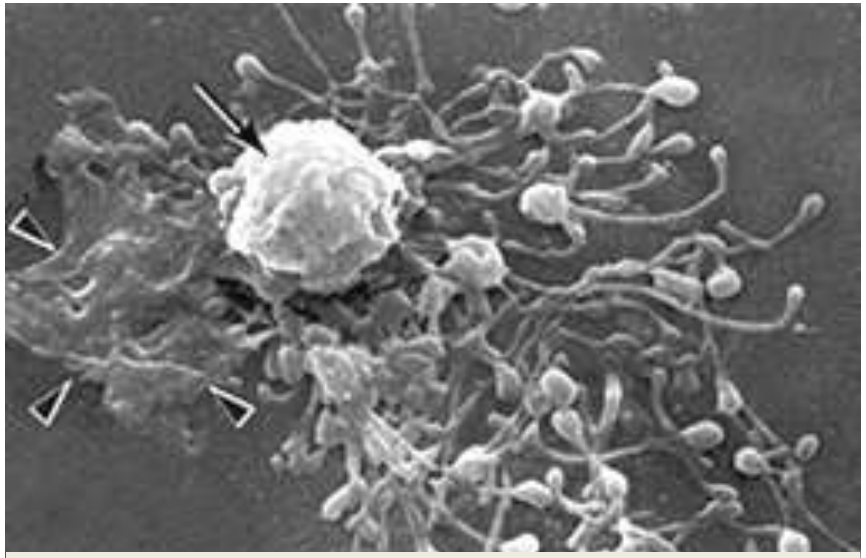


IC bound by FDCs are organized in a bead-like formation called as ‘iccosomes’

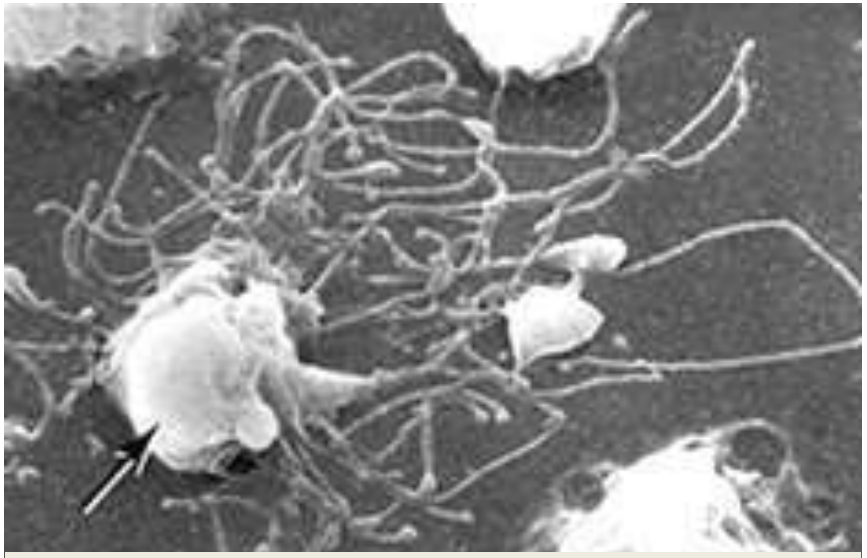
Association of antigen with FDC



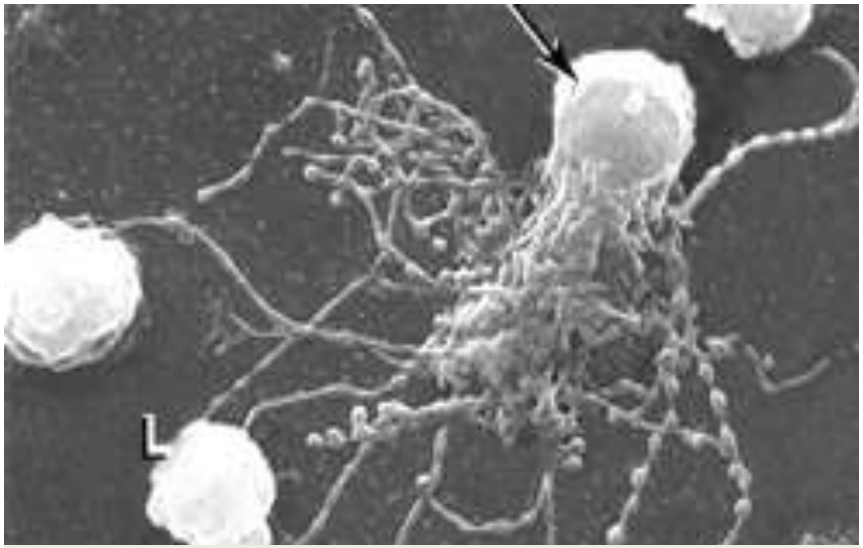
Maturation of Follicular Dendritic cells



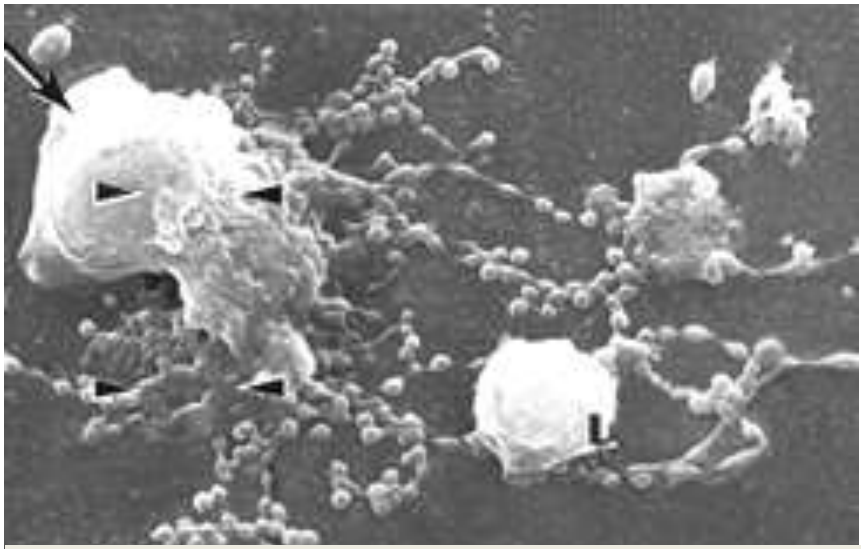
Club-shaped tips of developing dendrites



Filiform dendrites



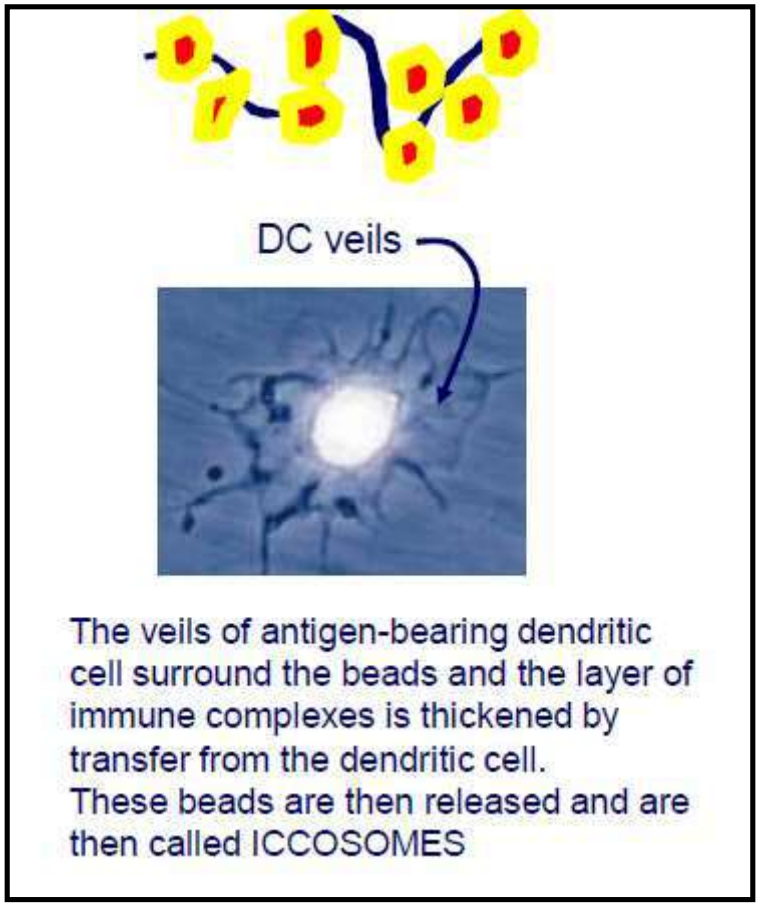
Bead formation on dendrites



Bead formation on dendrites

Iccosomes

- **Iccosomes (Ics)** are the immune-complex coated bodies formed when an antigen is injected into an immune animal. Theses are found in Dendritic cells. May serve as a reservoir of a antigen to maintain B-cell memory.

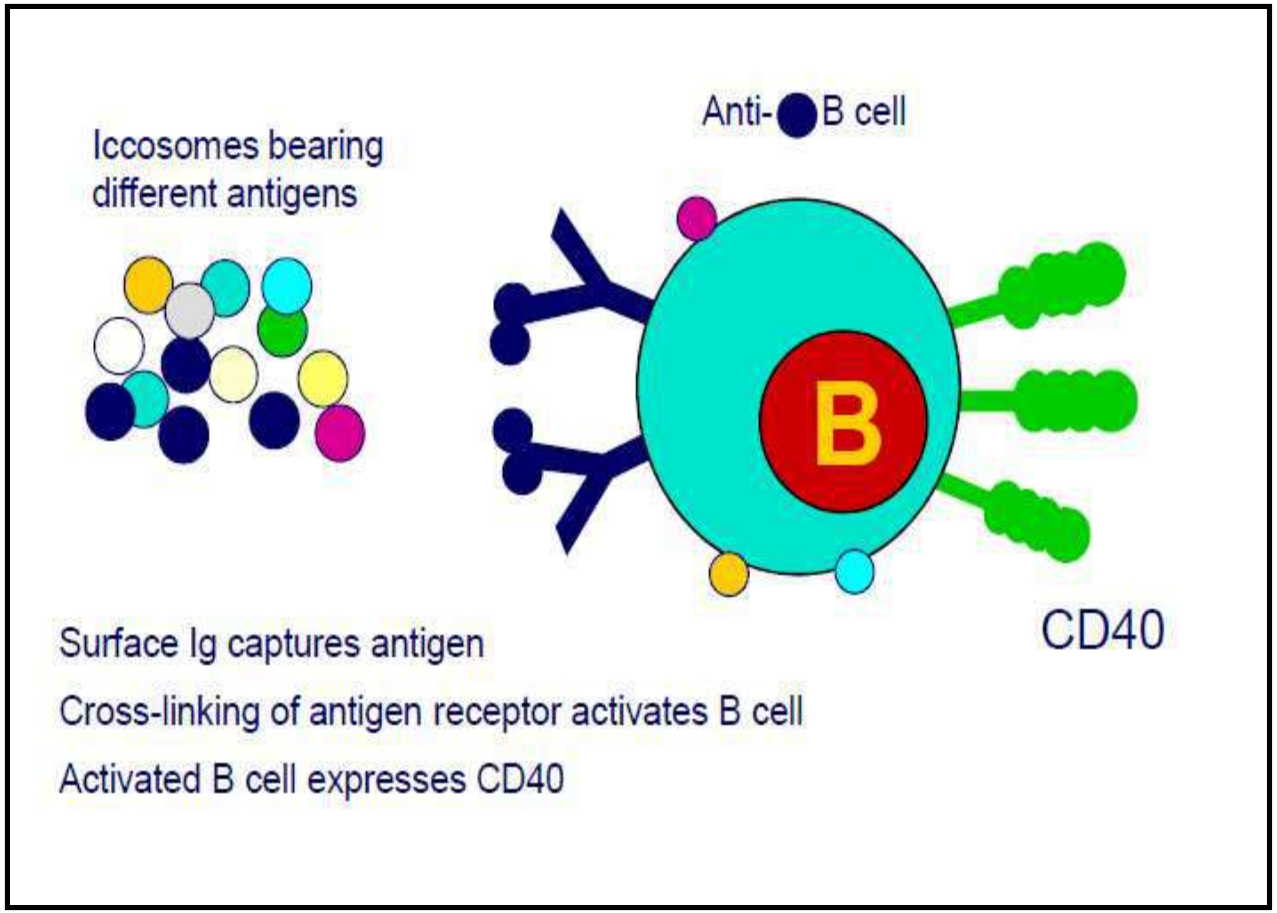


The veils of antigen-bearing dendritic cell surround the beads and the layer of immune complexes is thickened by transfer from the dendritic cell.
These beads are then released and are then called ICCOSOMES

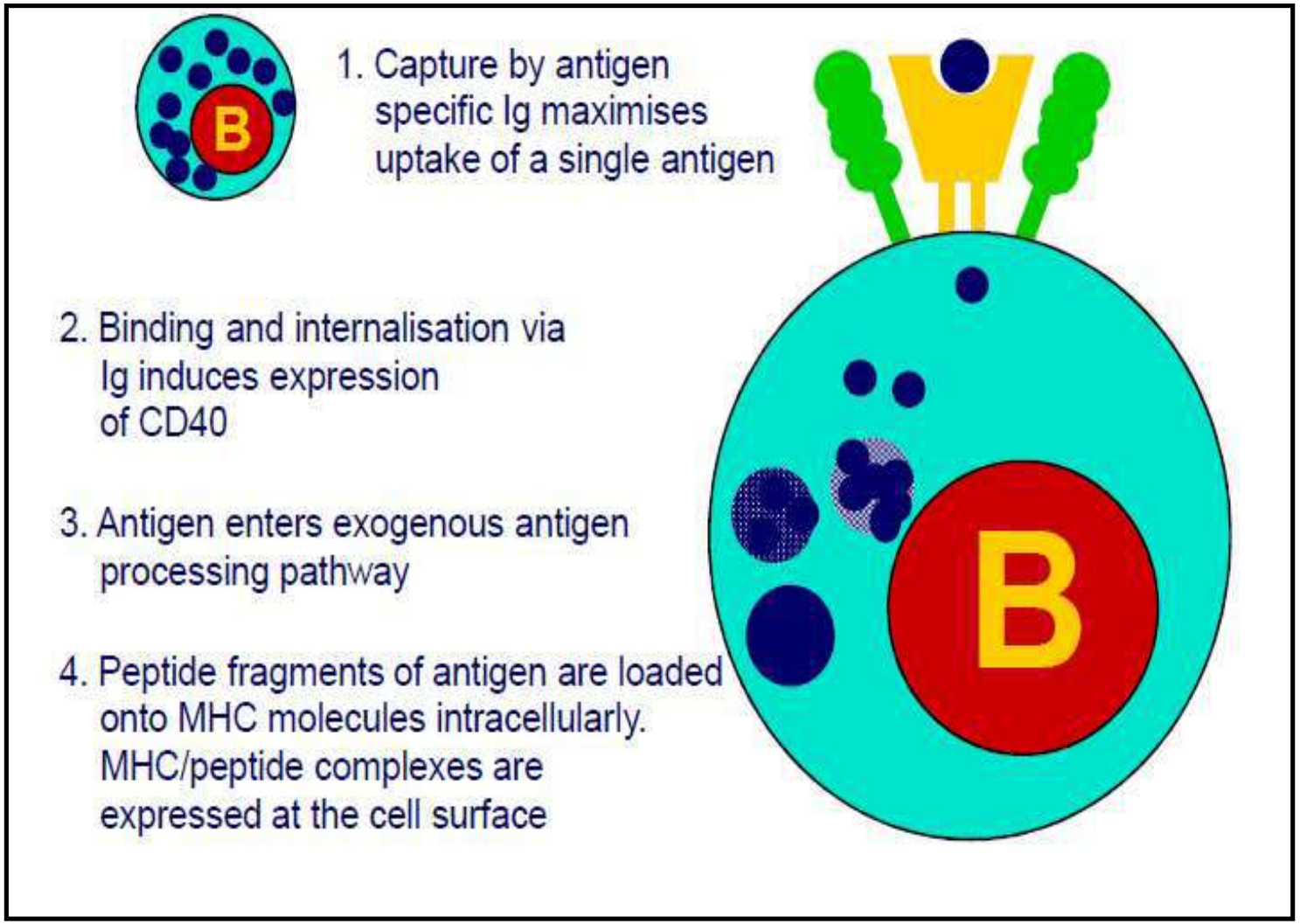
B LYMPHOCYTES

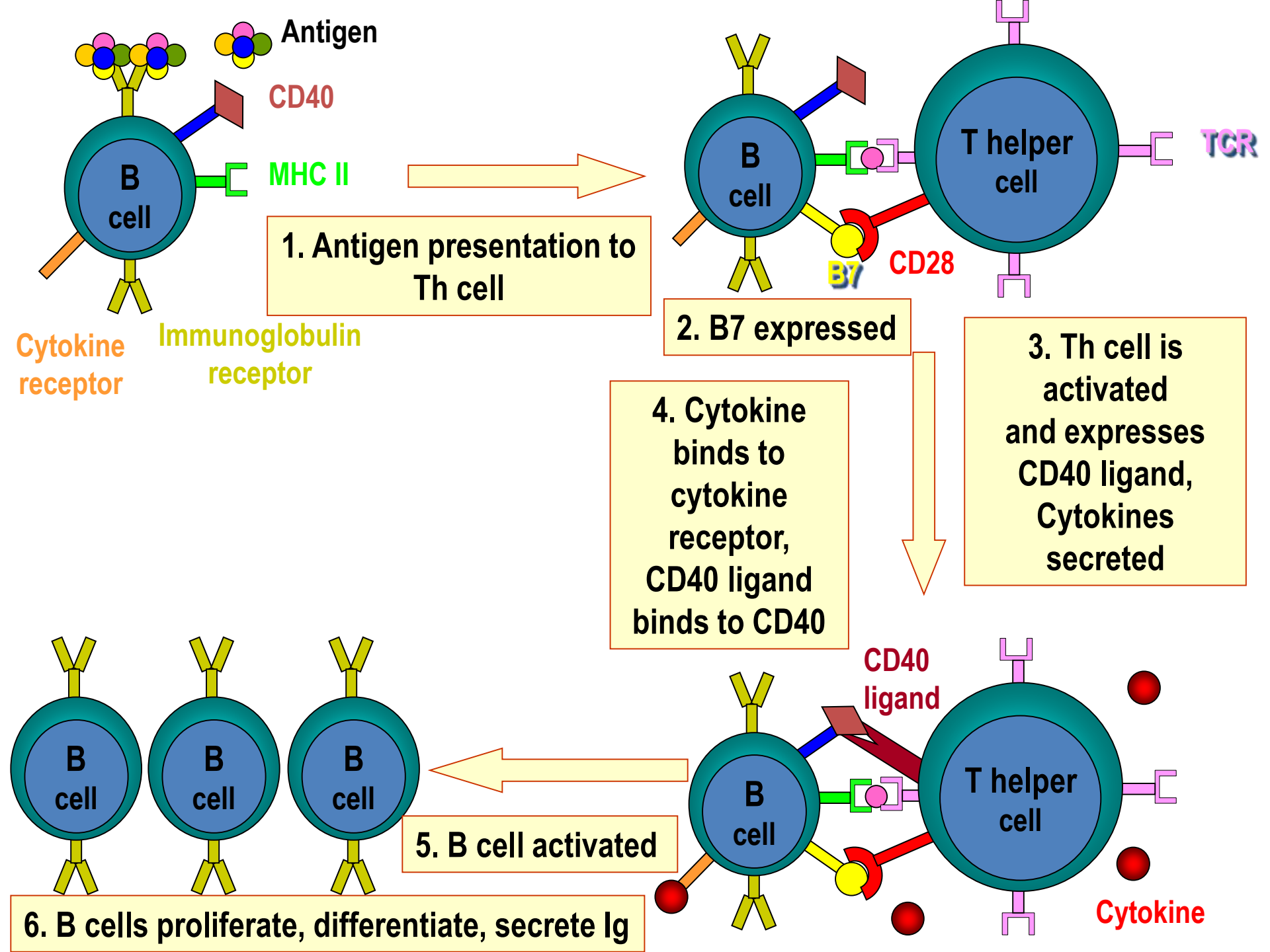
(as APC)

Uptake of Iccosomes/native Antigen by B cells



Processing and Presentation of internalised antigen




Antigen
CD40
B cell
MHC II
Cytokine receptor
Immunoglobulin receptor
1. Antigen presentation to Th cell
B cell
T helper cell
TCR
B7
CD28
2. B7 expressed
3. Th cell is activated and expresses CD40 ligand, Cytokines secreted
4. Cytokine binds to cytokine receptor, CD40 ligand binds to CD40
CD40 ligand
B cell
T helper cell
Cytokine
5. B cell activated
B cell
B cell
B cell
6. B cells proliferate, differentiate, secrete Ig