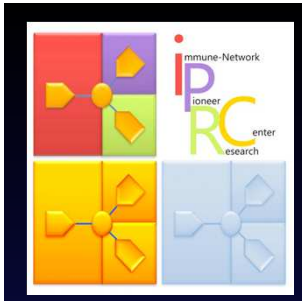


Defense of Cellular Damage by Link between Innate and Adaptive Immunity

Jong-Young Kwak, MD. PhD

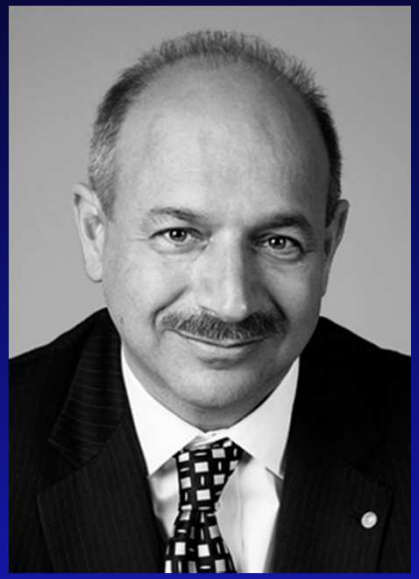
Department of Biochemistry, Dong-A University School of Medicine

Immune-network Pioneer Research Center

Busan, Korea

2013. 10. 24. Mendel University in Brno

The Nobel Prize in Physiology or Medicine 2011



Bruce A. Beutler

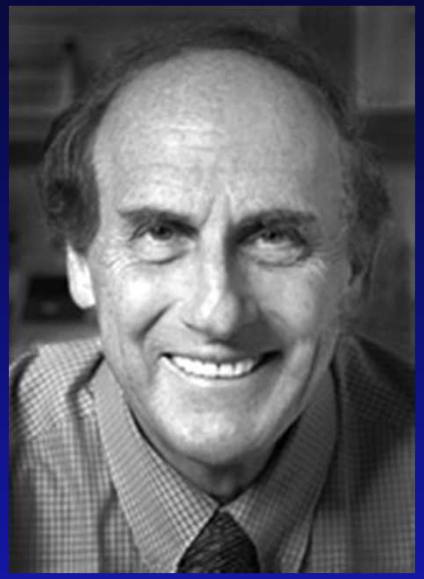
The Scripps Research Institute, La Jolla, CA, USA

Discoveries concerning the activation of innate immunity

Jules A. Hoffmann

University of Strasbourg, Strasbourg, France

Discoveries concerning the activation of innate immunity



Ralph M. Steinman

Rockefeller University, New York, NY, USA

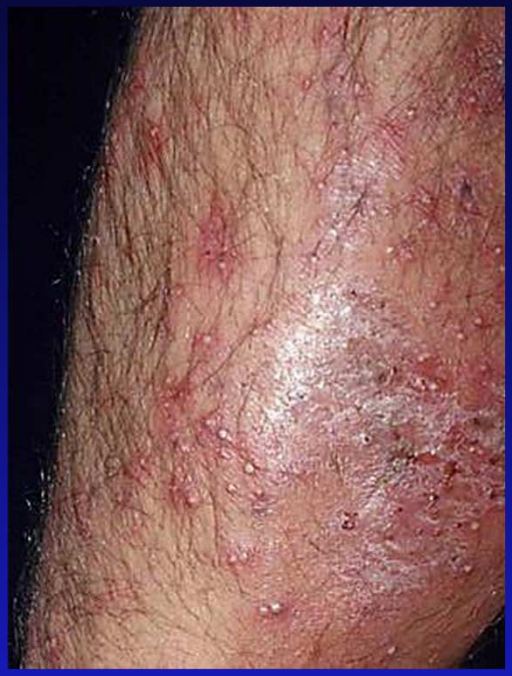
Discoveries of the dendritic cell and its role in adaptive immunity

Contents

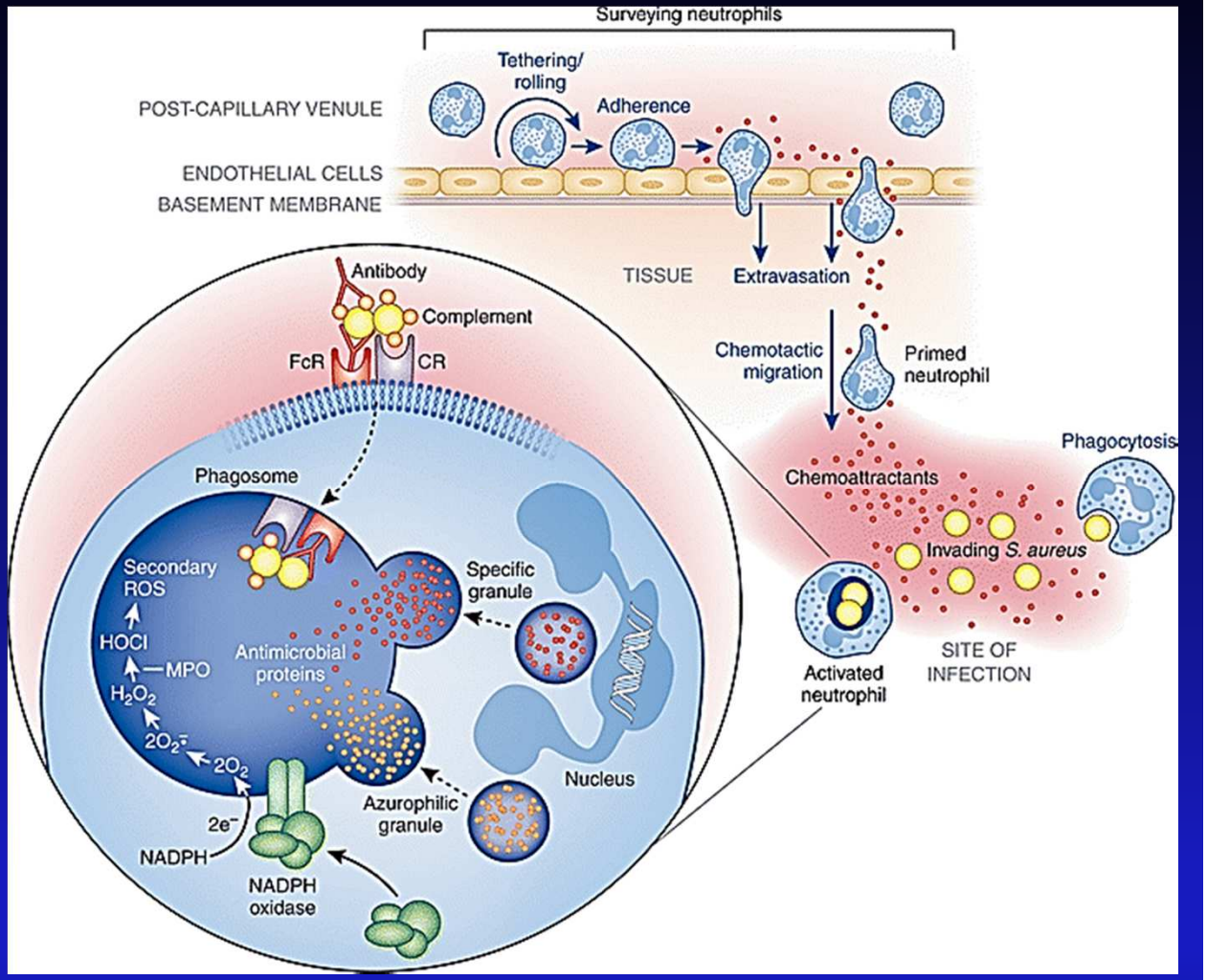
1. Innate Immunity – Bacterial Skin Infection and Body's Defense
2. From Innate to Adaptive Immunity – Cytokine Production
3. Link to Adaptive Immunity against Exogenous Pathogens
4. Link to Adaptive Immunity against Self-modified Antigens

1. Innate Immunity – Bacterial Skin Infection and Body's Defense

● *Staphyococcus aureus* Folliculitis

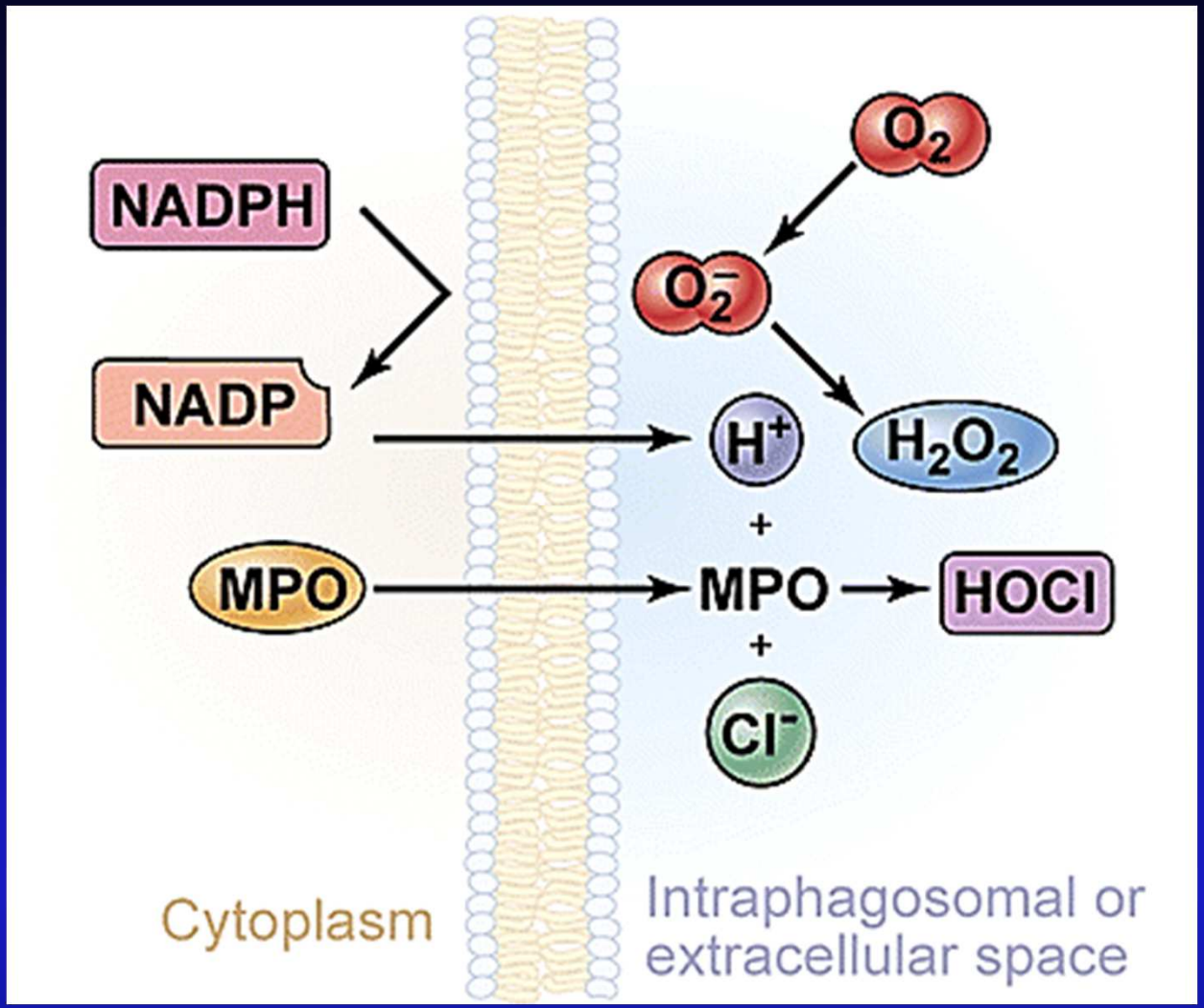


- ❖ erythematous
- ❖ warm
- ❖ edematous papules
- ❖ pustules

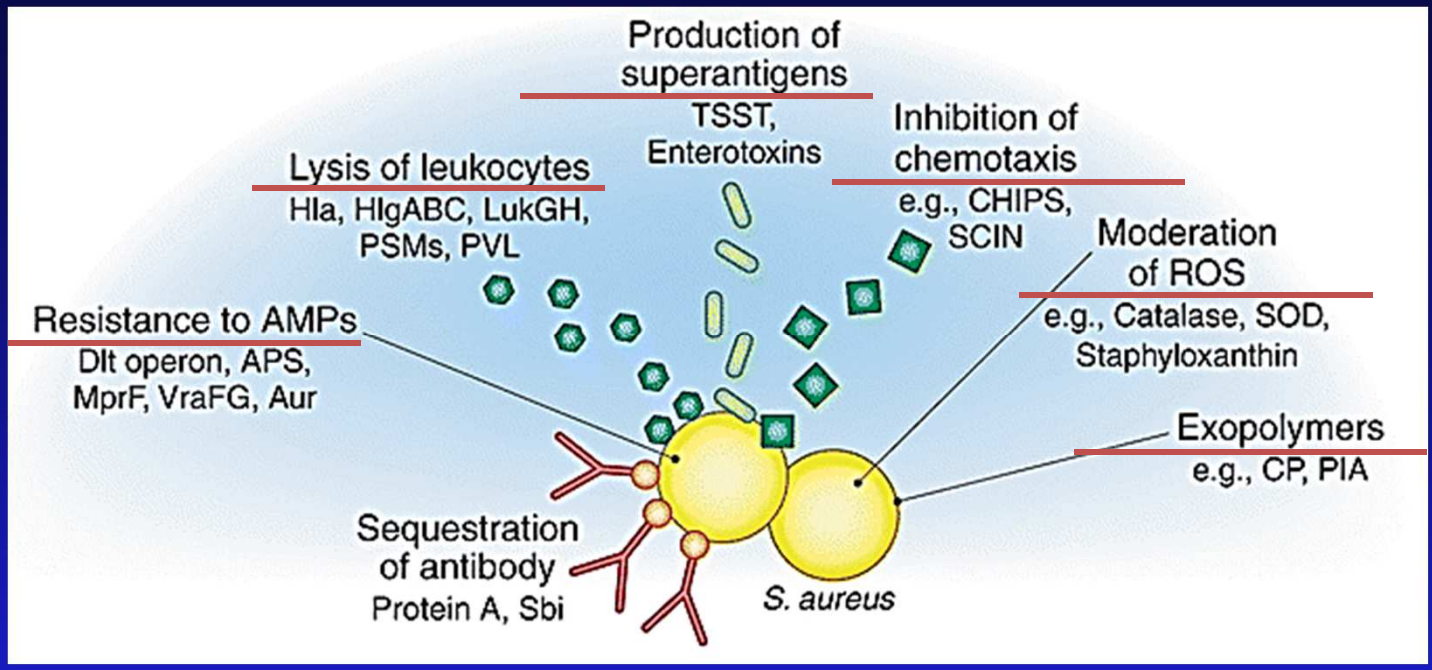


- ● Neutrophil (polymorphonuclear leukocytes, PMNLs) emigration from vascular space to site of infection, phagocytosis and microbial killing

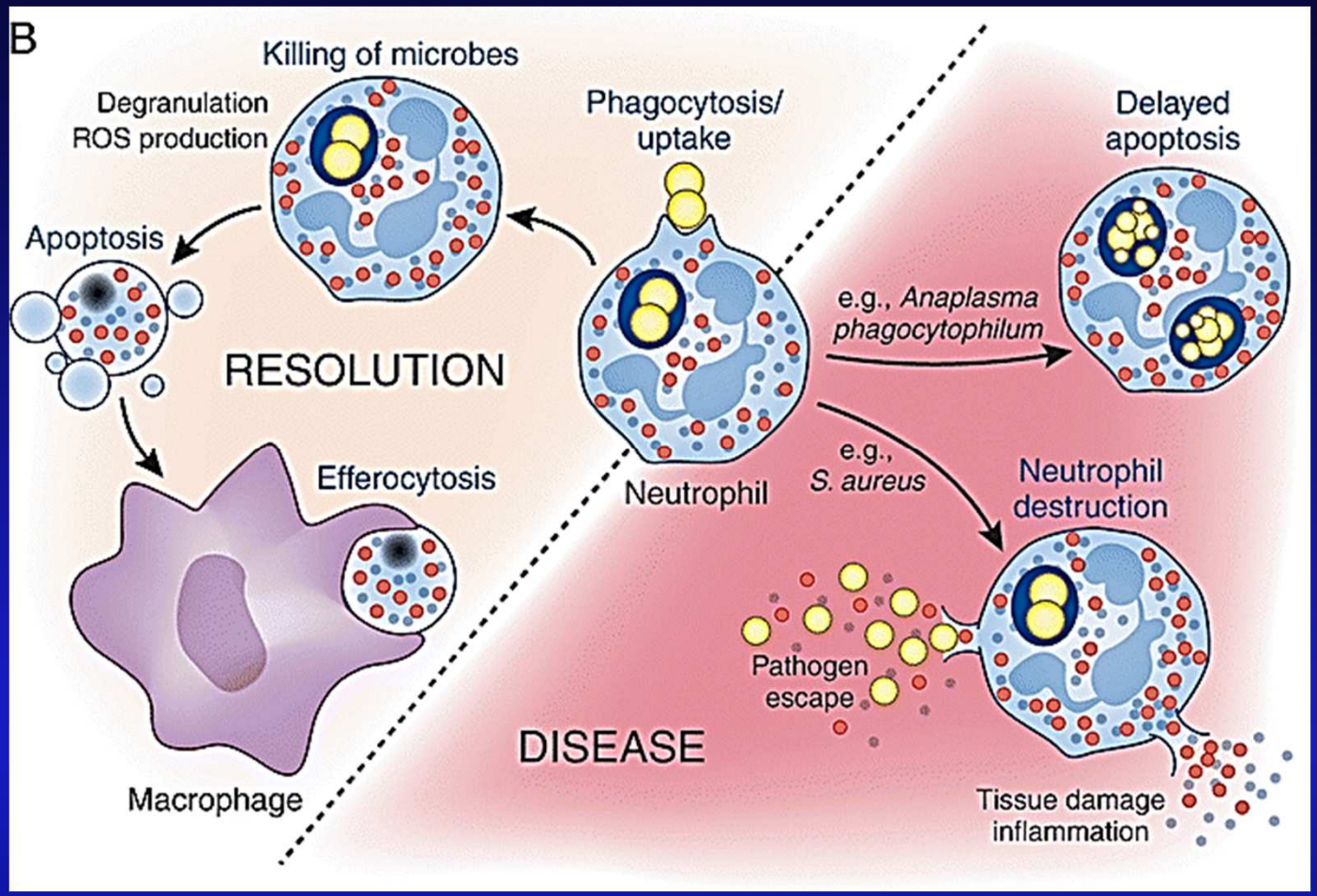
MPO-H_2O_2-Chloride Antimicrobial System



Immune Evasion Mechanisms by Bacteria



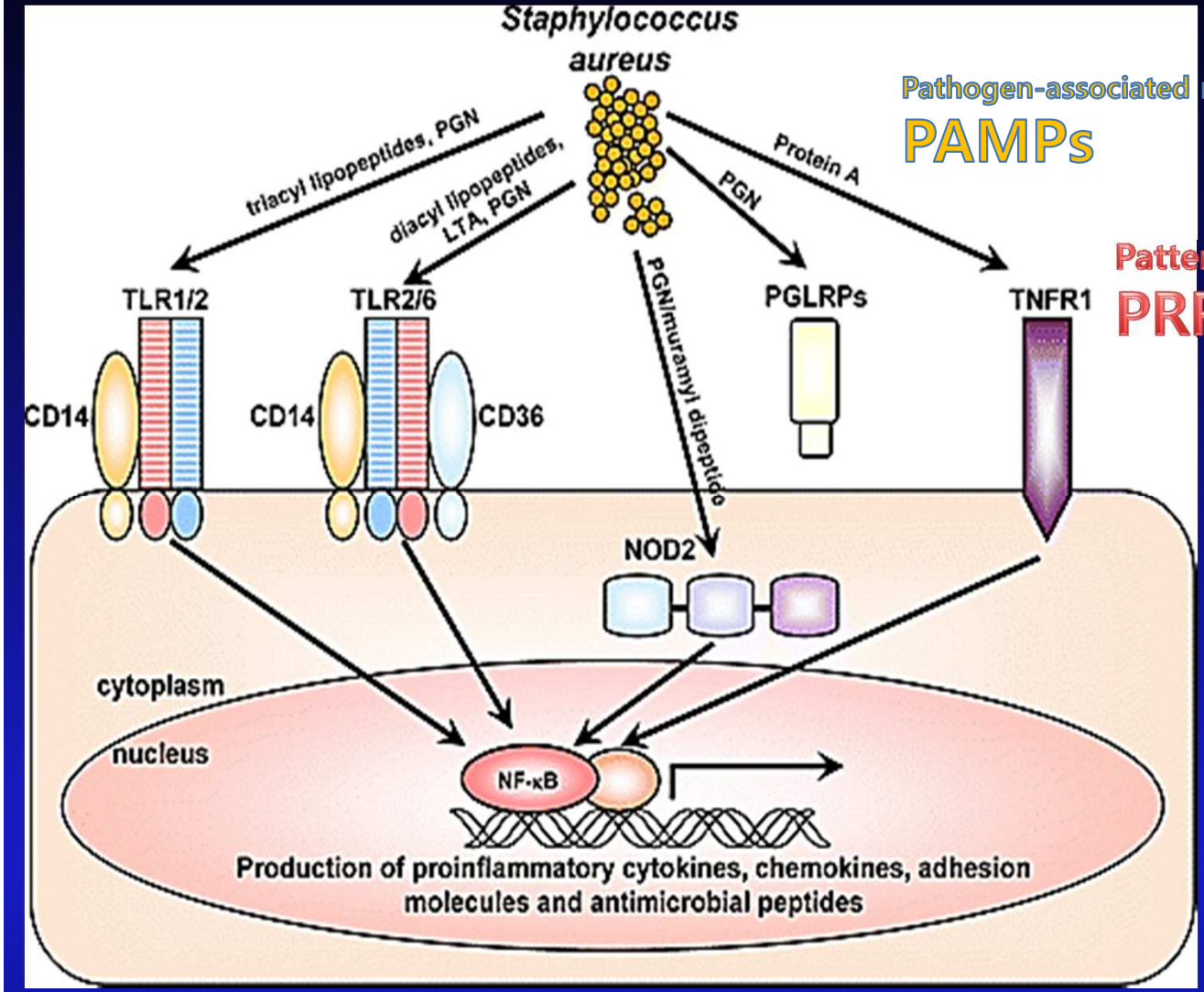
Possible Outcomes of Bacteria–Neutrophil Interaction



1. Innate Immunity – Bacterial Skin Infection and Body's Defense

2. **From Innate to Adaptive Immunity – Cytokine Production**

Pattern Recognition Receptors (PRRs) Recognizing *S. aureus*



Pathogen-associated molecular pattern

PAMPs

Pattern recognition receptors

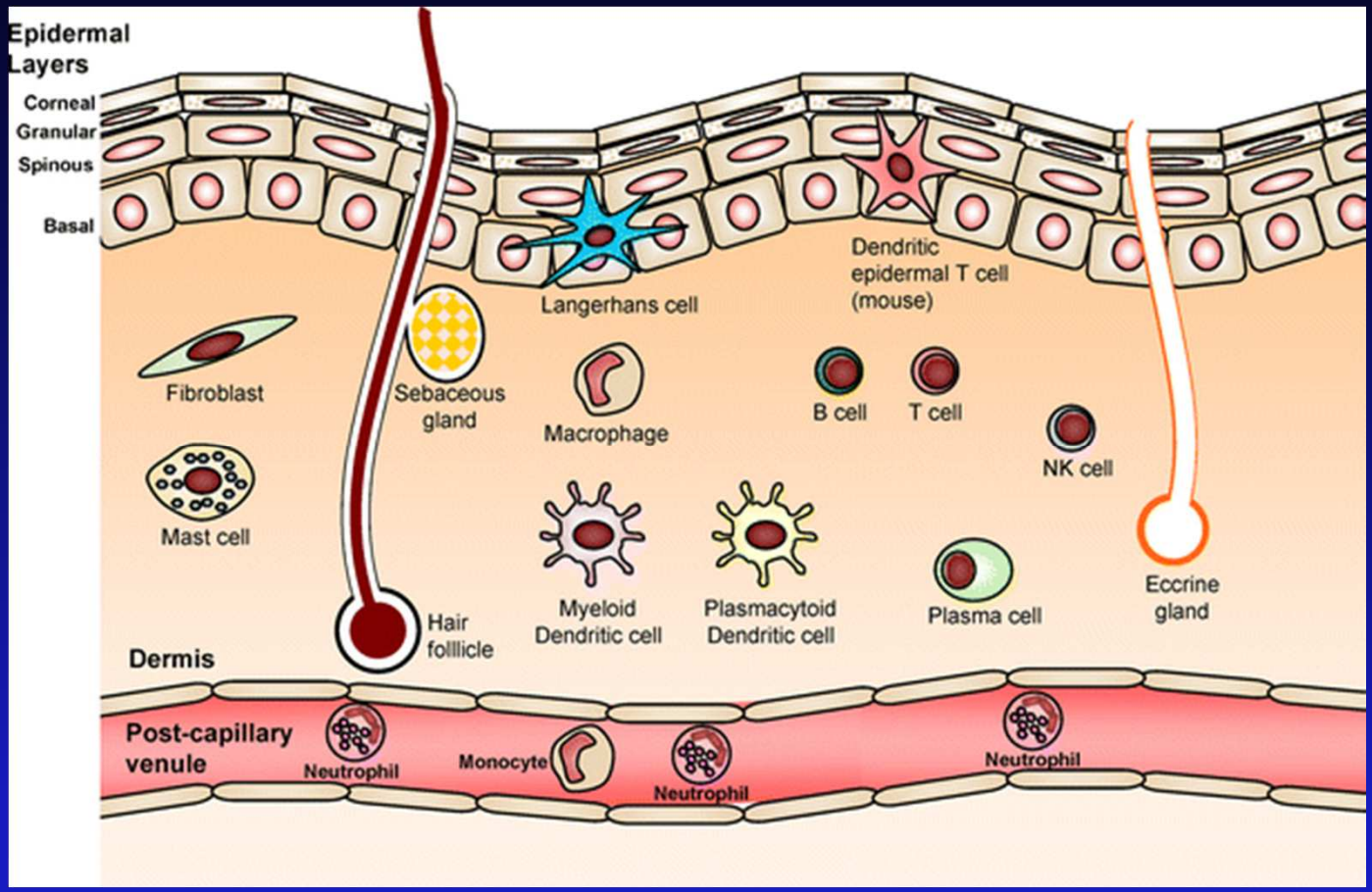
PRRs

Toll-like receptors (TLRs)

Peptidoglycan receptor proteins (PGRPs or PGLYRPs)

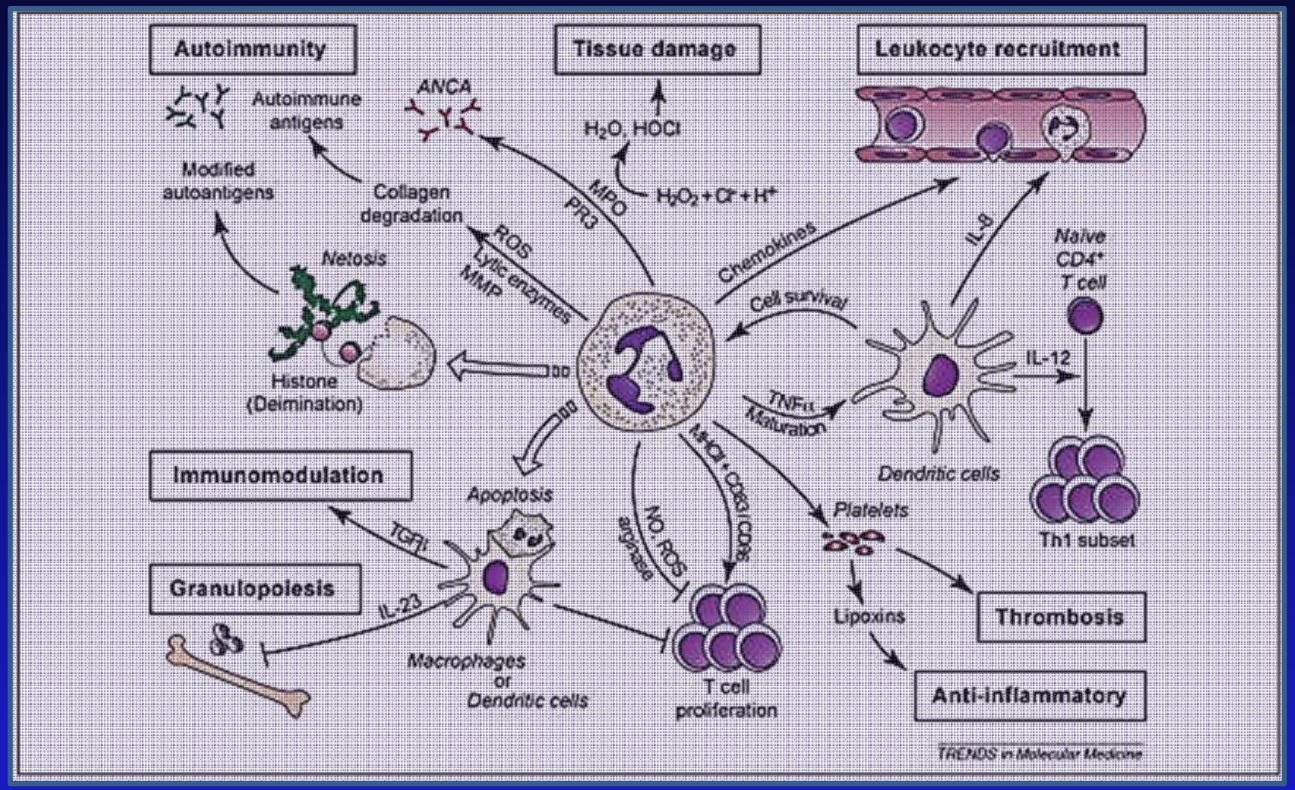
Nucleotide-binding oligomerization domain containing 2 (NOD2)

PRR-Expressing Cells Recognize *S. aureus*



Keratinocytes, Langerhans cells, monocytes/macrophages, dendritic cells, mast cells, endothelial cells, fibroblasts, and adipocytes

Neutrophils Shape the Immune Landscape

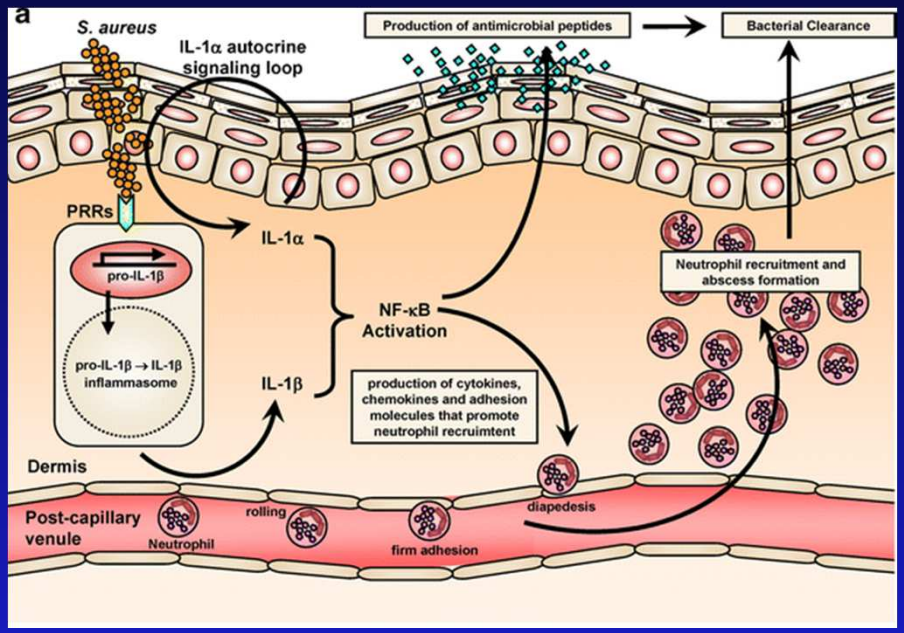


Innate Immune System Components

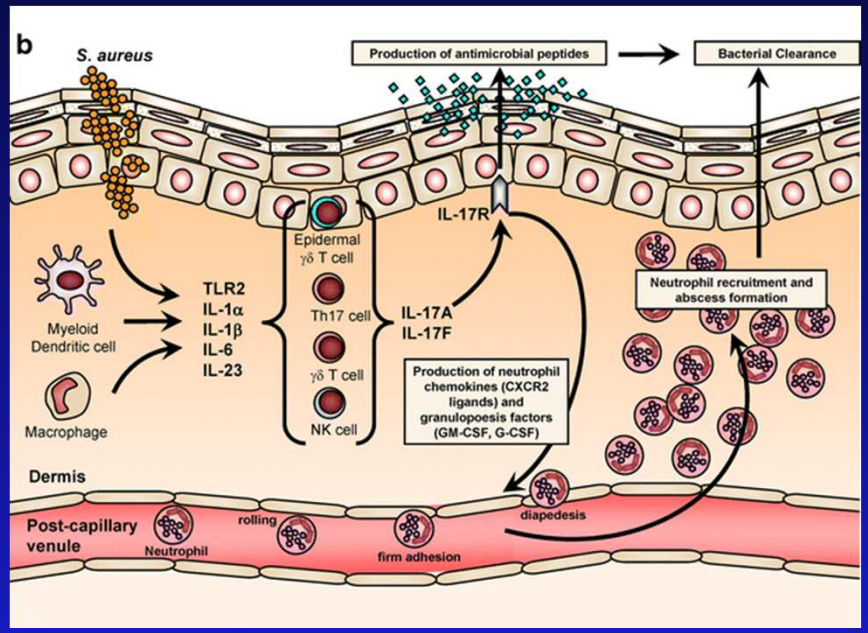
Natural barriers	Cells	Pattern-recognition receptors	Cytokines	Natural antimicrobial products
Skin,	Neutrophils,	Mannose-	IL-1, IL-6	Defensins
Mucosal	Macrophages	banding lectins,	IL-8, IL-12	Lactoferrin
epithelia	Dendritic cells,	TLRs	IL-15, IL-18,	Lysozyme
	Natural killer cells		G-CSF, M-CSF	Natural antibodies
	Natural killer T cells,		GM-CSF, TNF-α	Complement
	γδ T cells		IFN-γ, ...	ROS
	B1 lymphocytes			

Cytokine Responses that Promote Clearance of *S. aureus* Skin Infections

IL-1 response

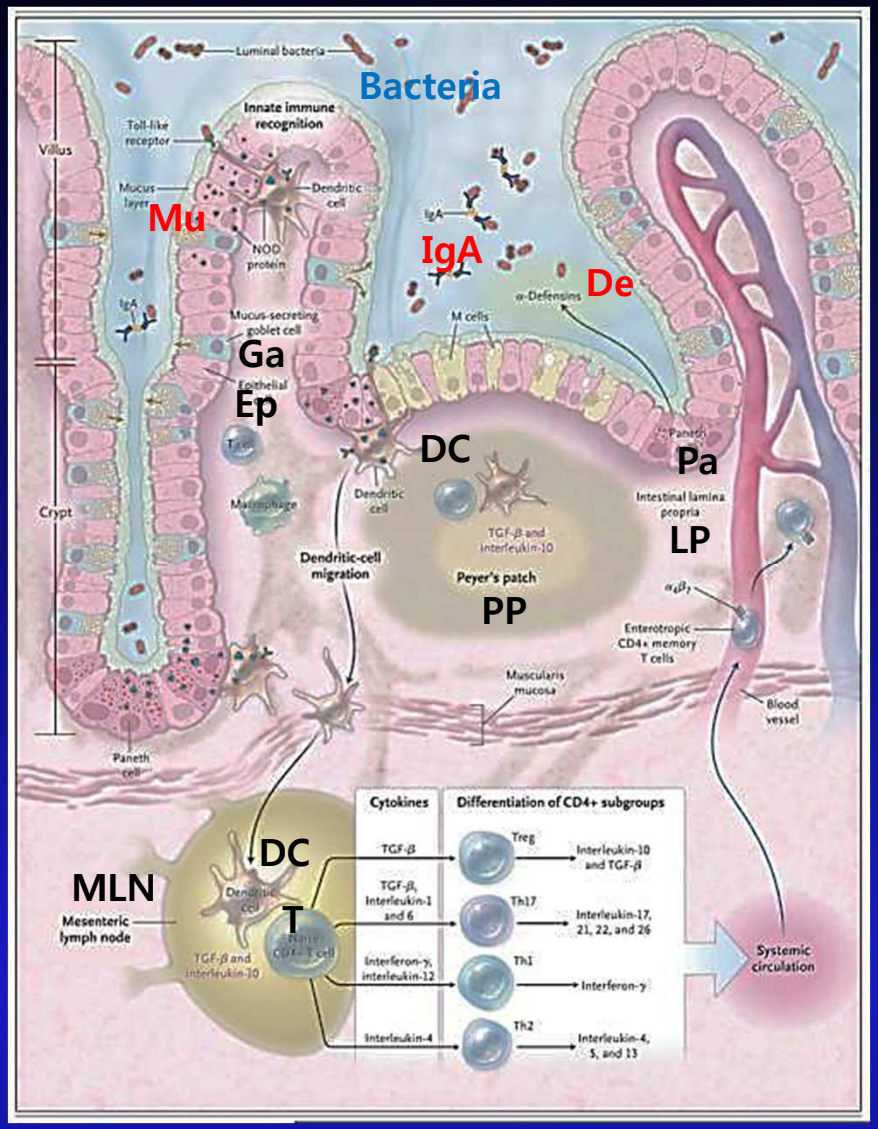


IL-17 response against *S. aureus*



Both the IL-1 and IL-17 responses promote neutrophil recruitment and abscess formation and keratinocyte production of antimicrobial peptides

The Intestinal Immune System in the Healthy State



goblet cells secrete a layer of mucus

α-defensins by Paneth cells

the production of IgA

microbial sensing by epithelial cells, dendritic cells, and macrophages

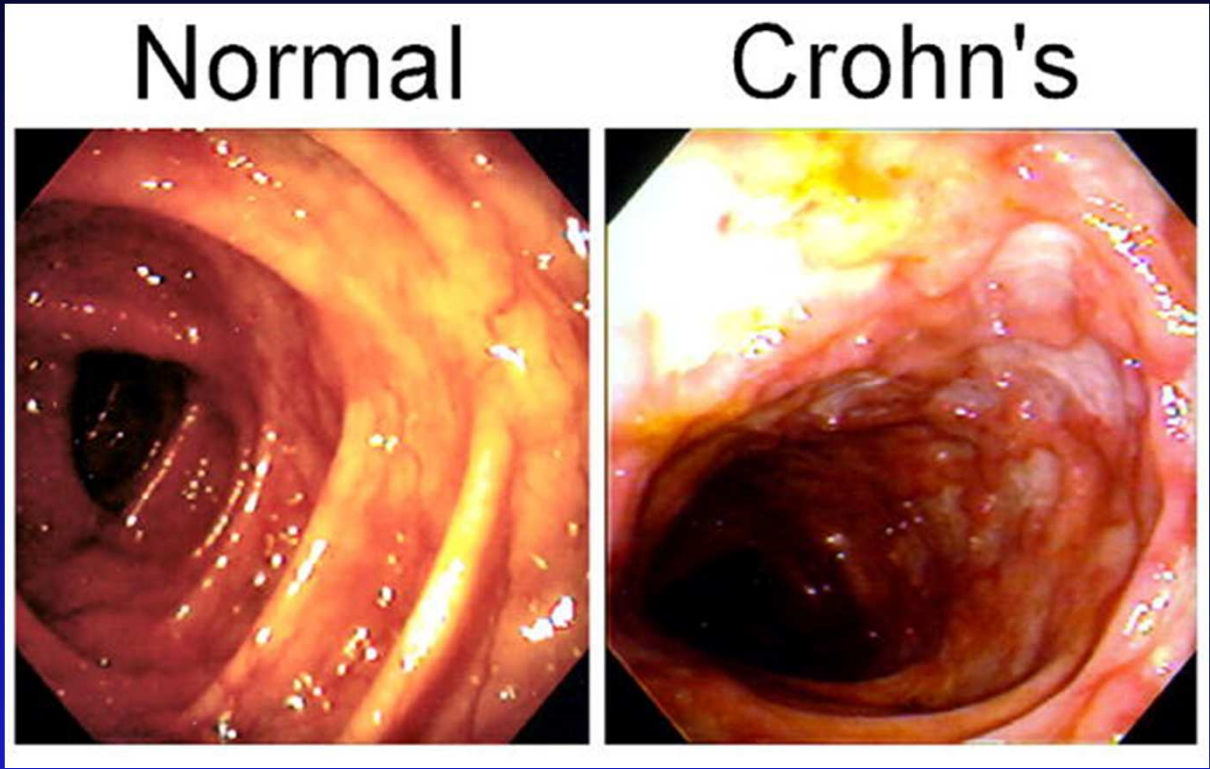
naive CD4+ T cells in secondary lymphoid organs (Peyer's patches and mesenteric lymph nodes)

characteristic cytokine profiles

activated CD4+ T cells then circulate to the intestinal lamina propria

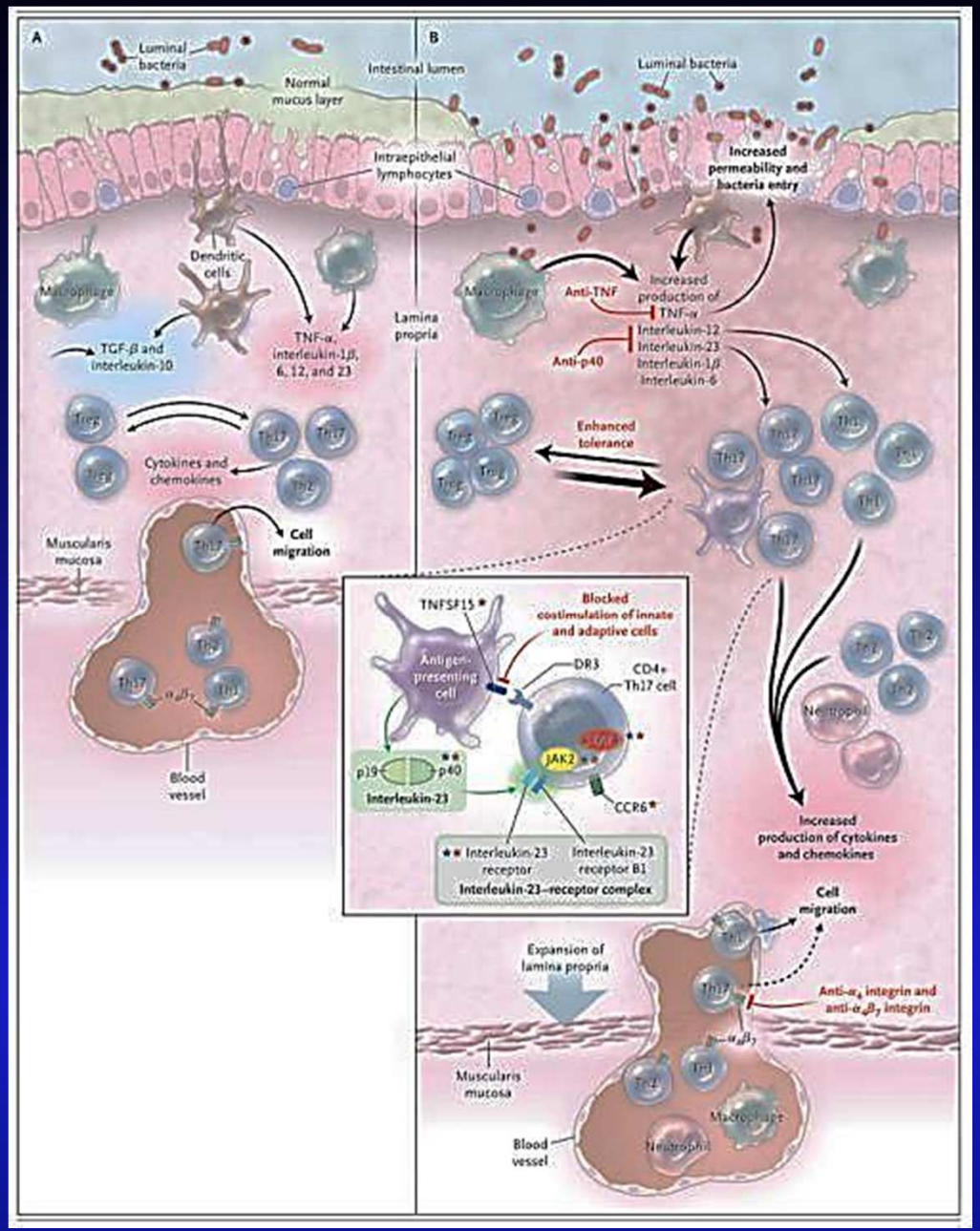
N Engl J Med. 2009; 361(21): 2066–207

Colonoscopic View of the Transverse Colon in Health (Normal) and Disease (Crohn's)



Clin Immunol. 2009 July; 132(1): 1–9.

The Intestinal Immune System in Crohn's Disease



Failure of bacterial elimination

Increased bacterial exposure

Increased proinflammatory cytokines (TNF-α, IL-23, IL-17)

Increased CD4⁺ T cells (Th-17)

Increased proinflammatory cytokines

Cycle of inflammation

1. Innate Immunity – Bacterial Skin Infection and Body's Defense
2. From Innate to Adaptive Immunity – Cytokine Production
3. **Link to Adaptive Immunity against Exogenous Pathogens**

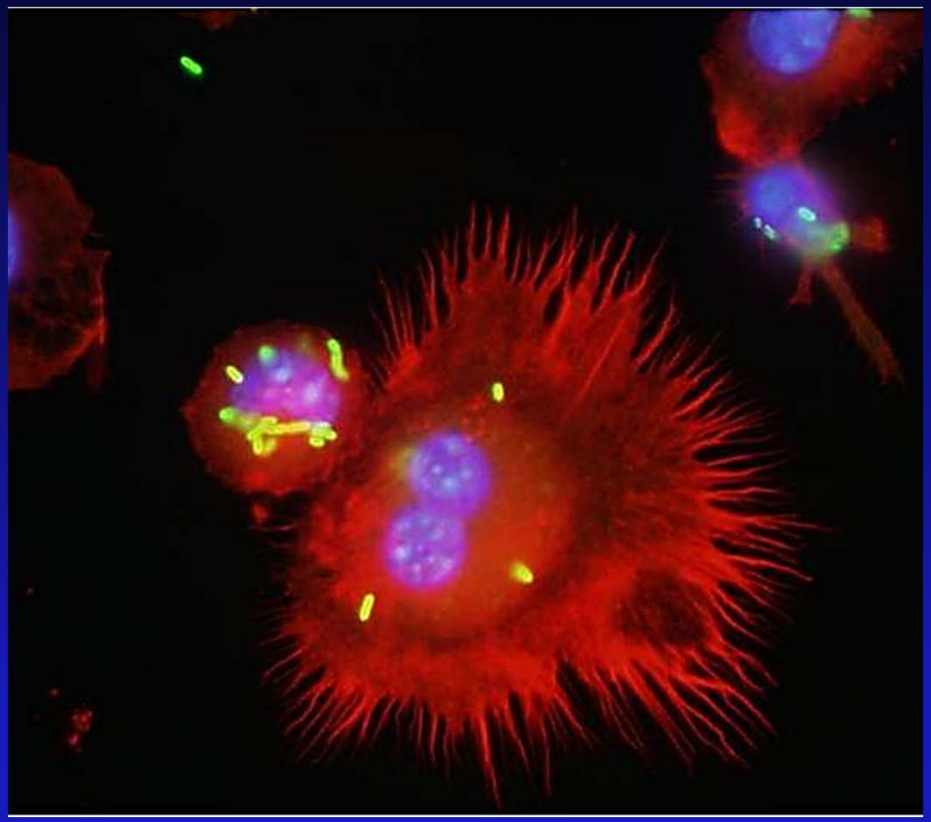
The adaptive immune response provides the vertebrate immune system with the ability to recognize and remember specific pathogens

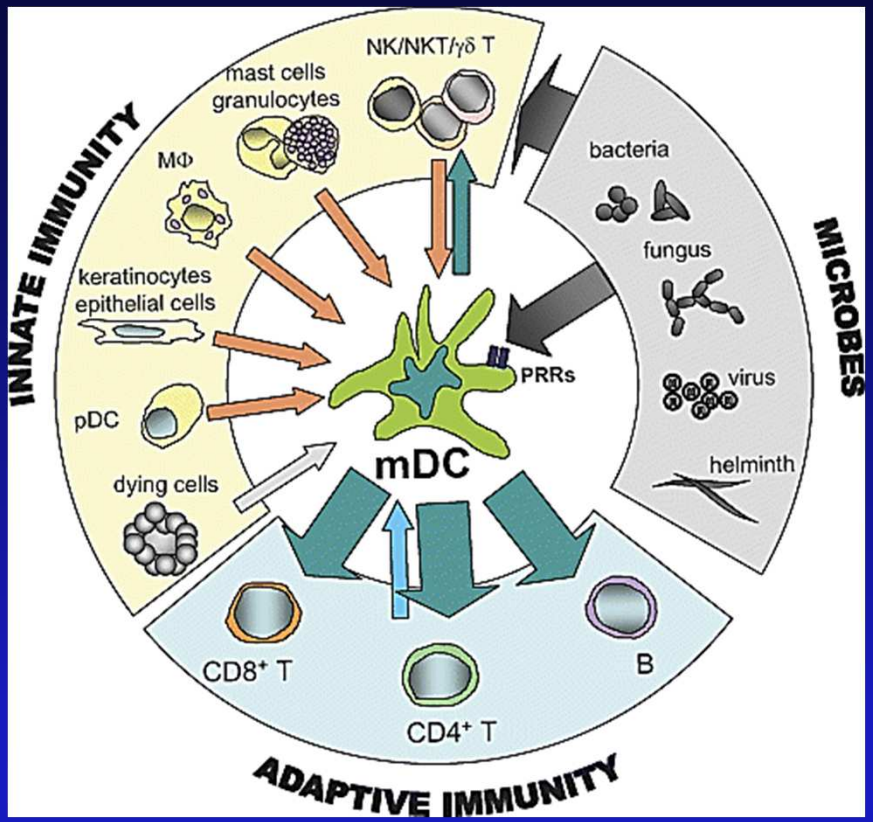
The cells of the adaptive immune system are a type of leukocytes, called a lymphocyte, B cells and T cell are the major types of lymphocytes.

T lymphocytes cannot scan the whole body searching for pathogens

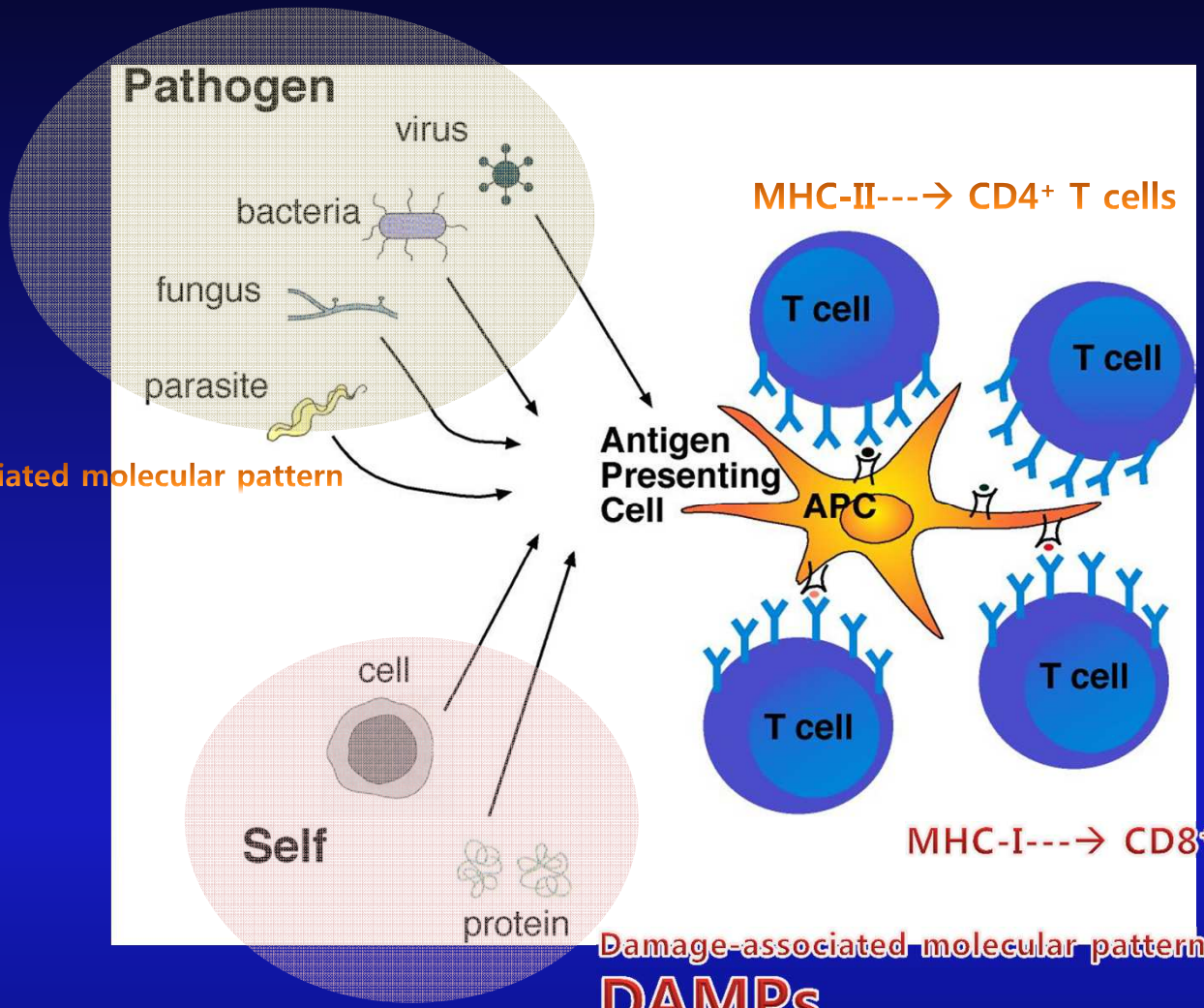
We need a "communication link" between the periphery and the lymph nodes

Dendritic Cells Link Innate Immunity to Adaptive Immunity

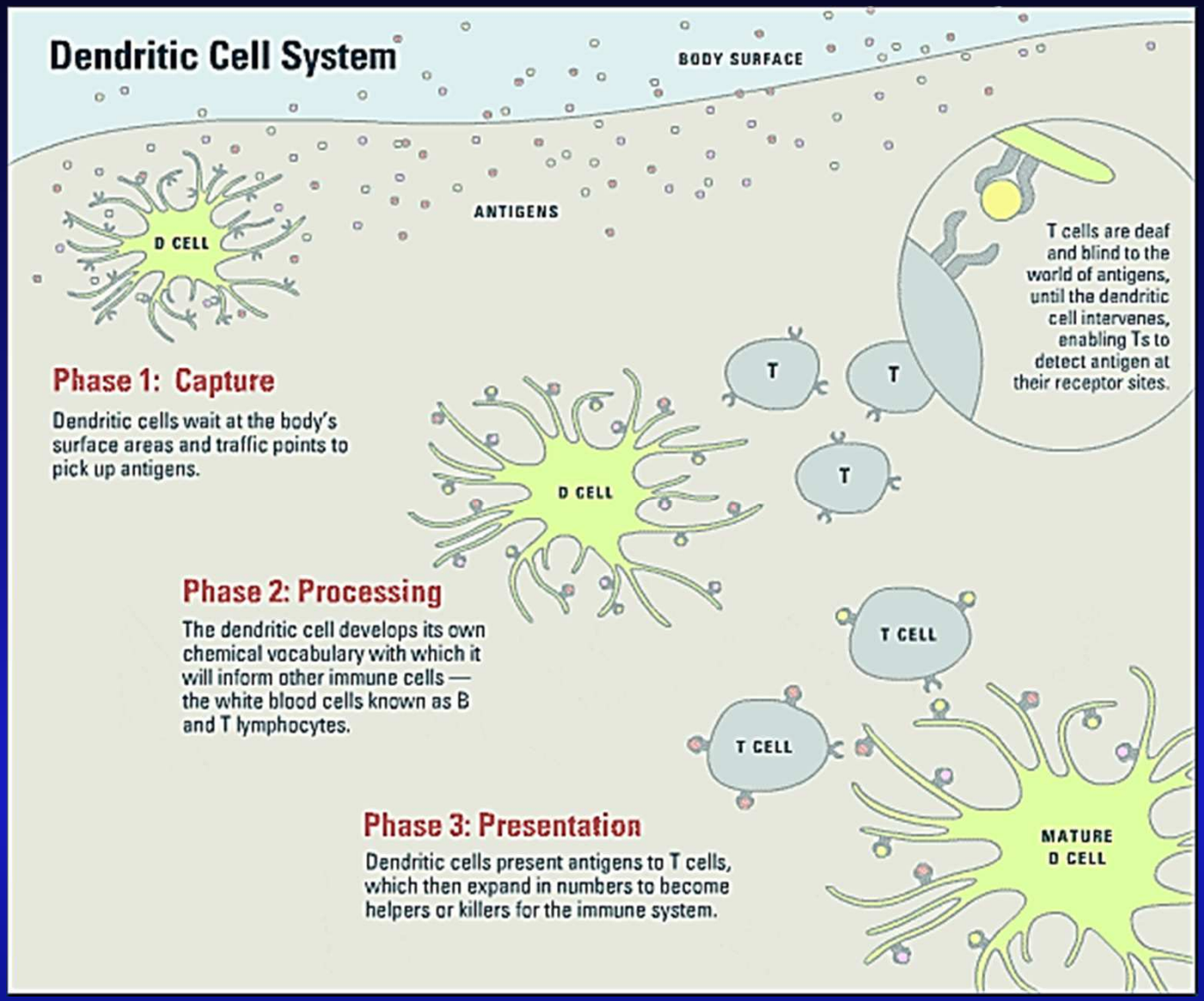




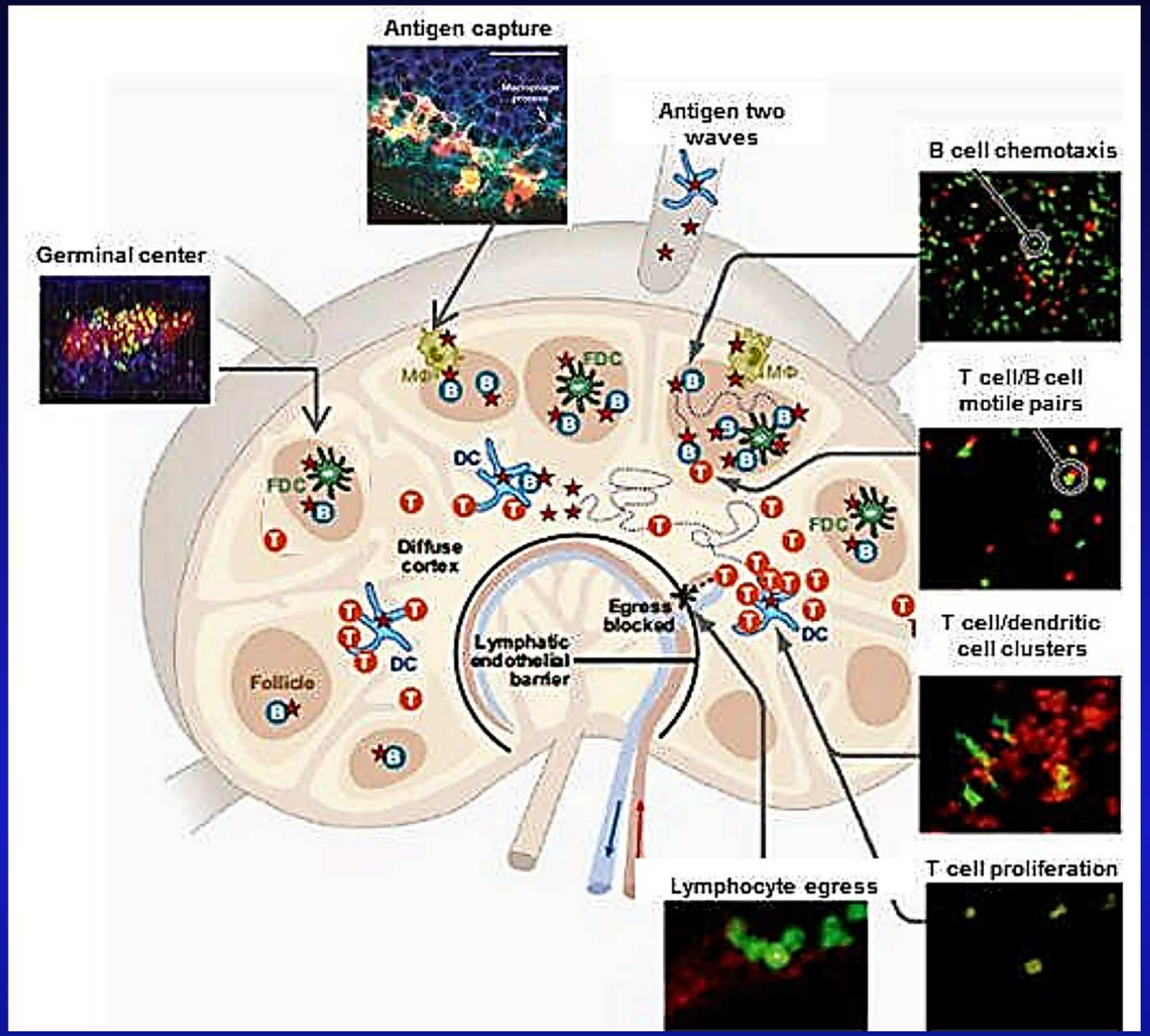
Dendritic Cells as Antigen-Presenting Cells



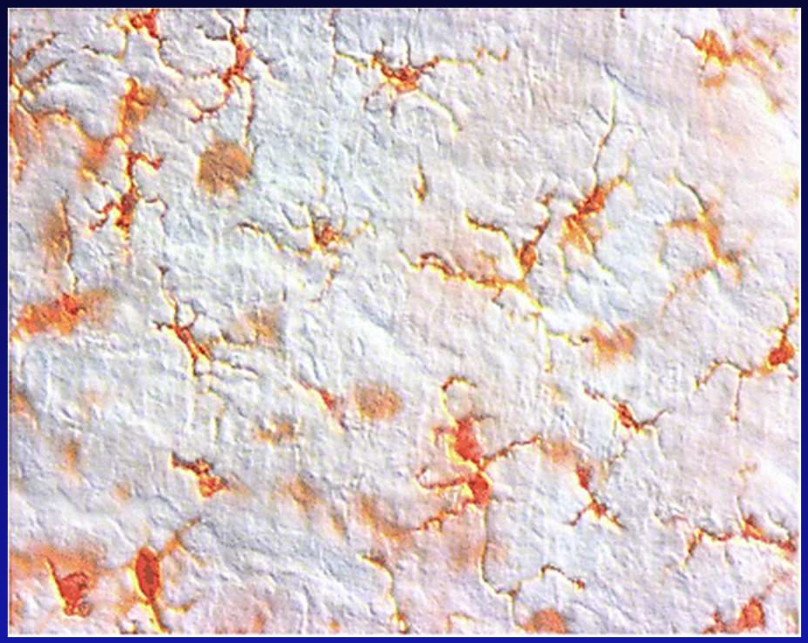
Antigen Capture, Processing and Presentation by Dendritic Cells



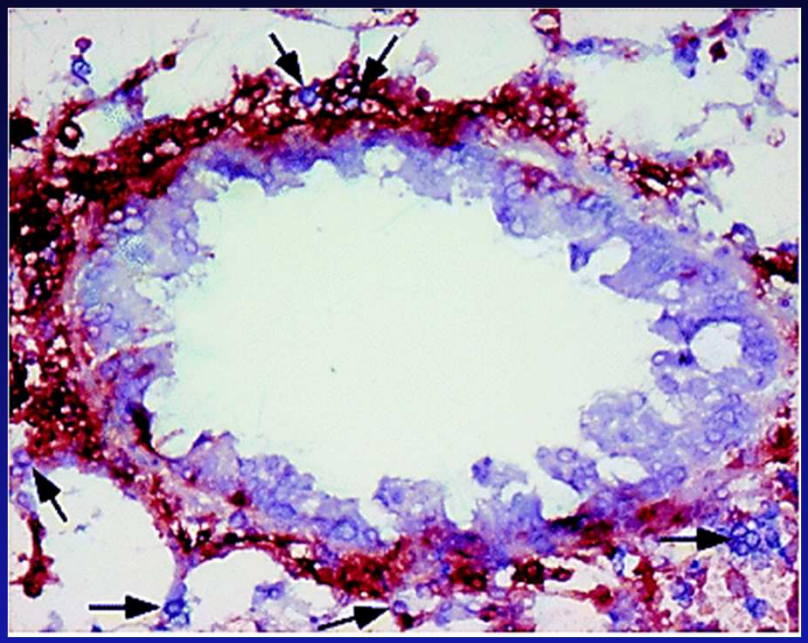
Lymph Node Cellular Choreography in Response to Antigen



Dendritic Cells as Sentinel Cells in Immune System

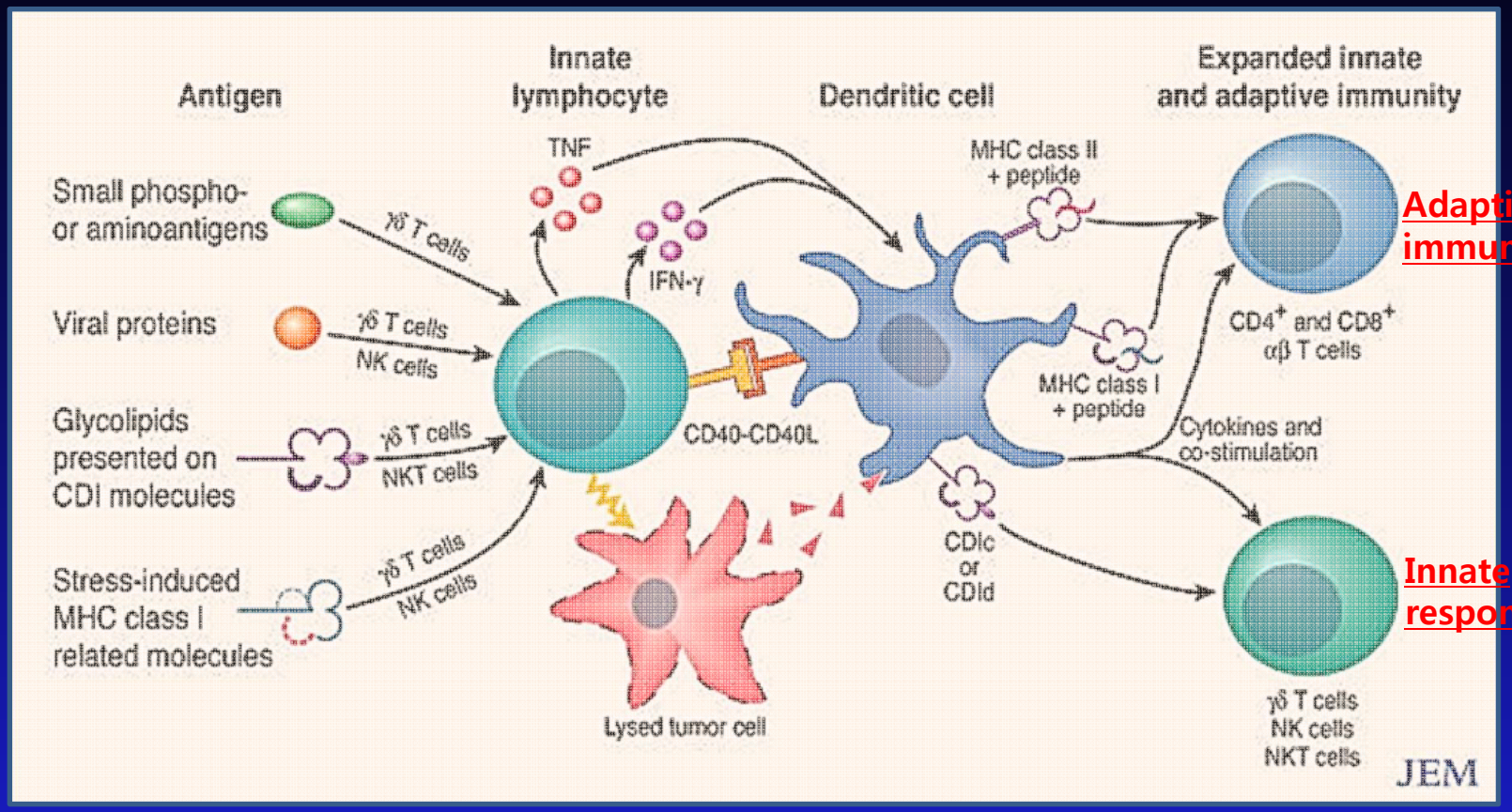


Mucosal dendritic cell network visualized by major histocompatibility complex II staining on a murine tracheal wholemount. Trachea was taken from a naïve not-immunized mouse.



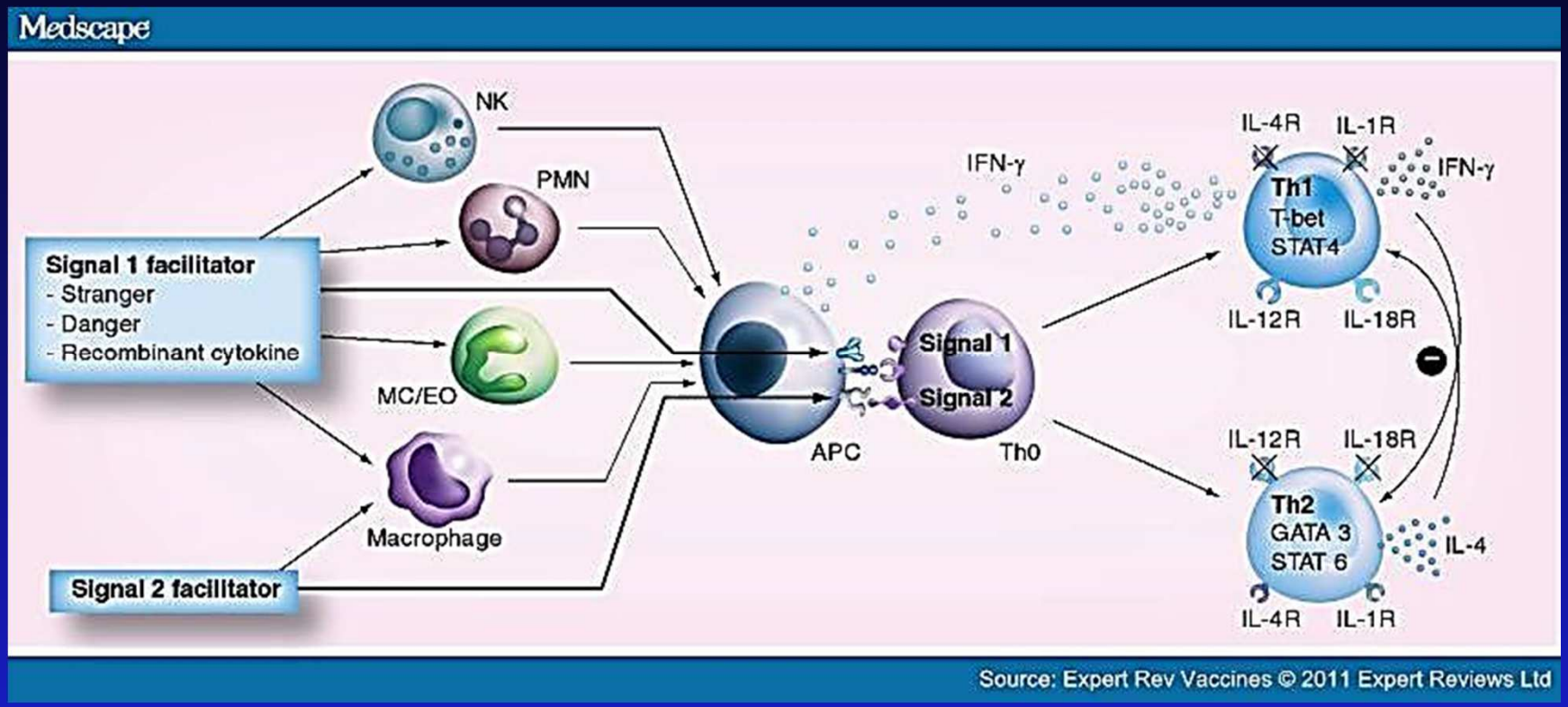
Staining of CD4+ T cells (membrane-bound blue; see arrows) and CD11c+ dendritic cells (DCs) (red) in the lung of ovalbumin-sensitized and challenged mice shows co-localization of CD4+ T cells and CD11c+ DCs within peribronchial sites of inflammation. Goblet cell hyperplasia is seen

Innate Lymphocytes Mature DCs



- Cytokines and cell contact–dependent molecules mediate DC activation
- DCs produce cytokines that expand and differentiate additional innate and adaptive lymphocytes.

Cytokine Production in Activated DCs



Cytokine production by dendritic cells affects the function of T cells

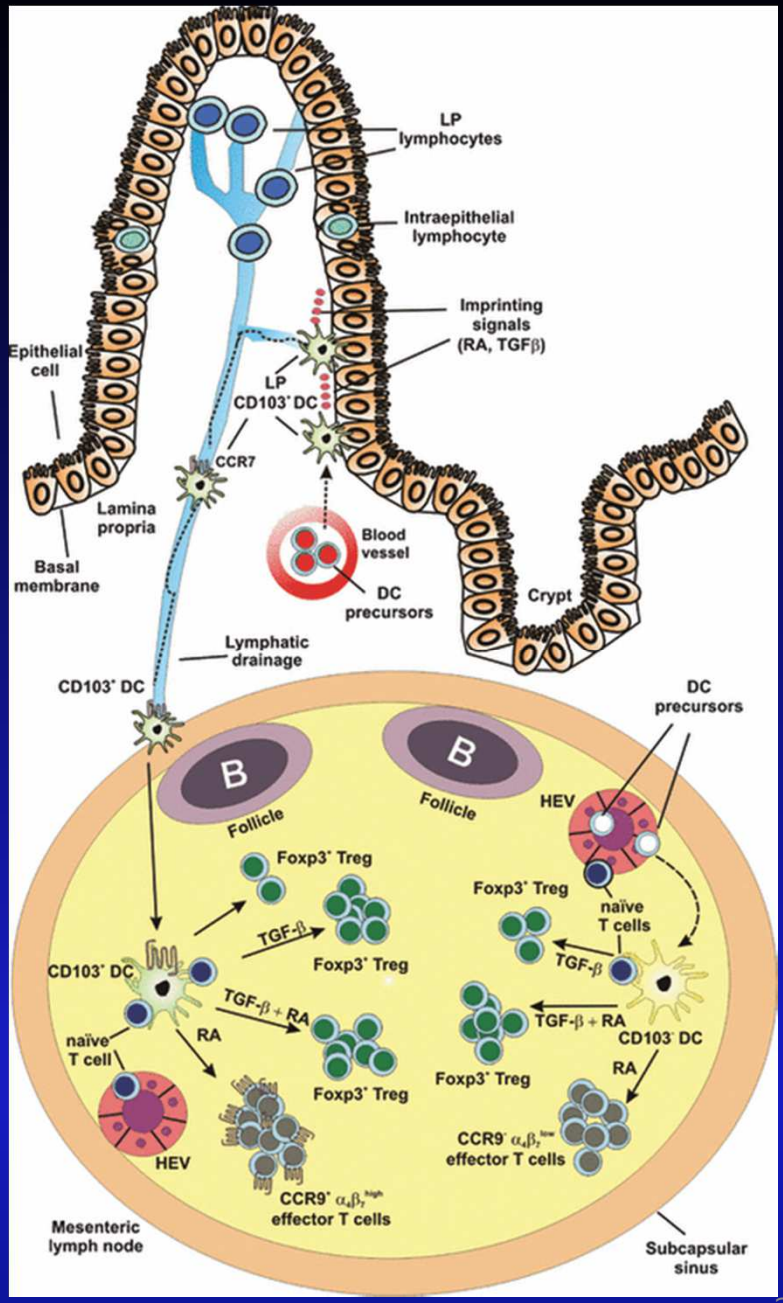
The Role of Regulatory Dendritic Cells in Intestine: Immune Tolerance

Regulatory dendritic cells

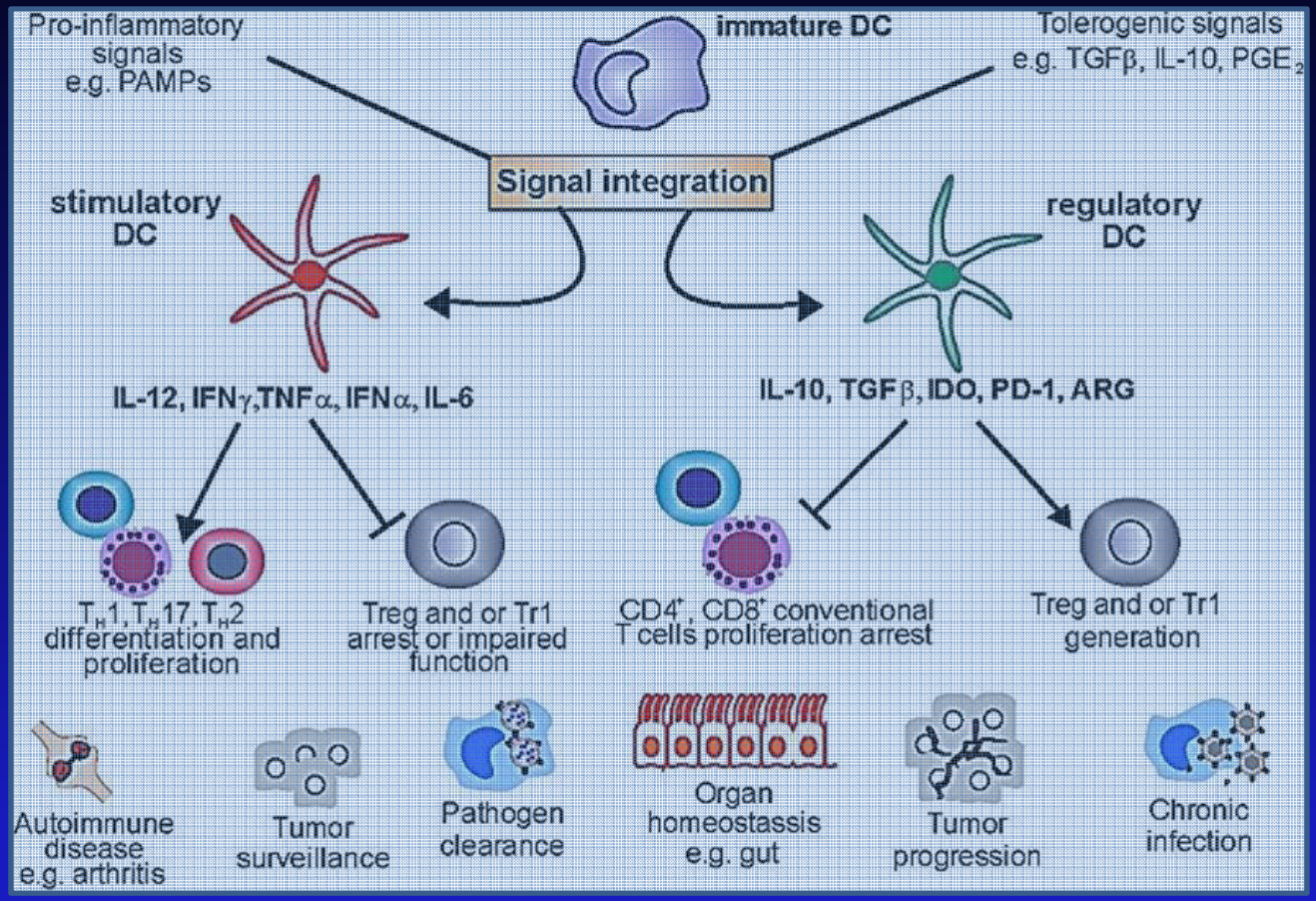
⬇

Regulatory T cells (Treg)

TGF-β !



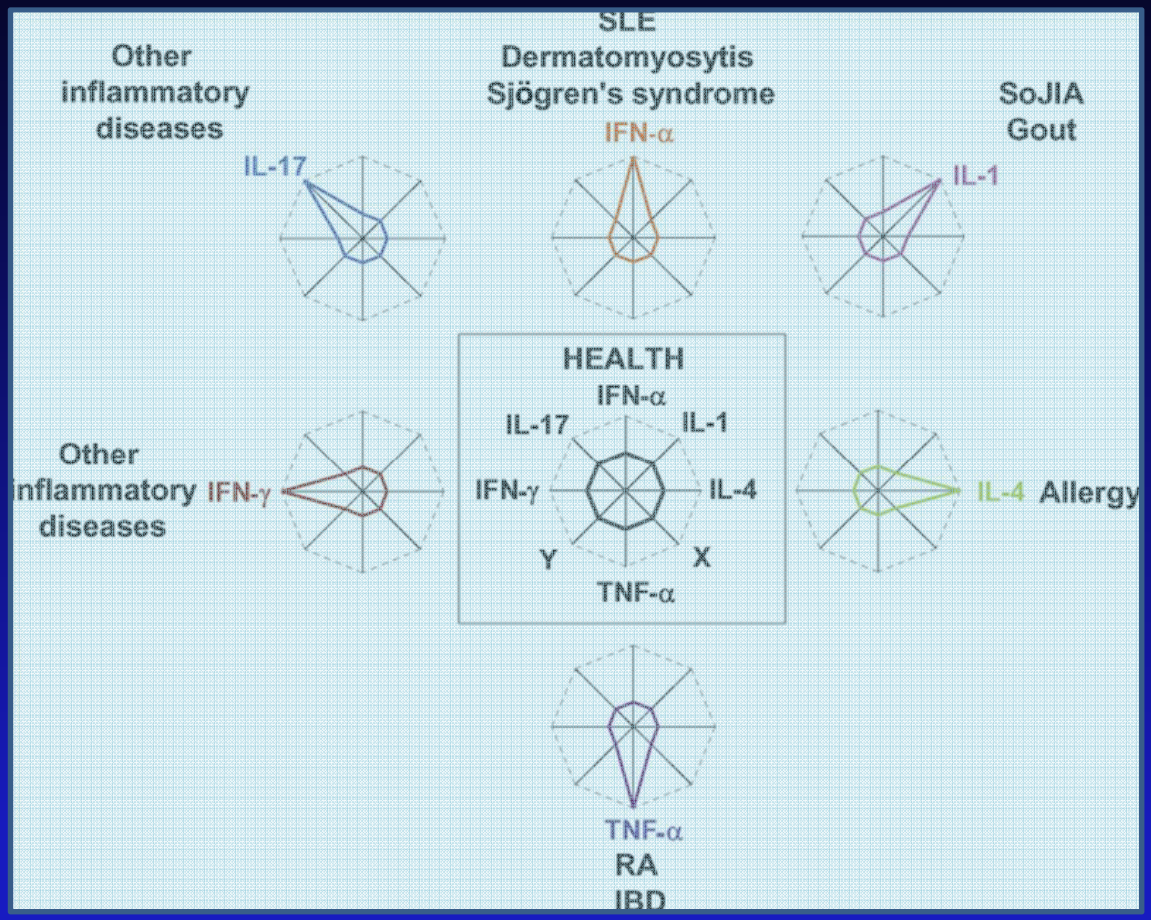
Stimulatory and Regulatory Dendritic Cells in Health and Diseases



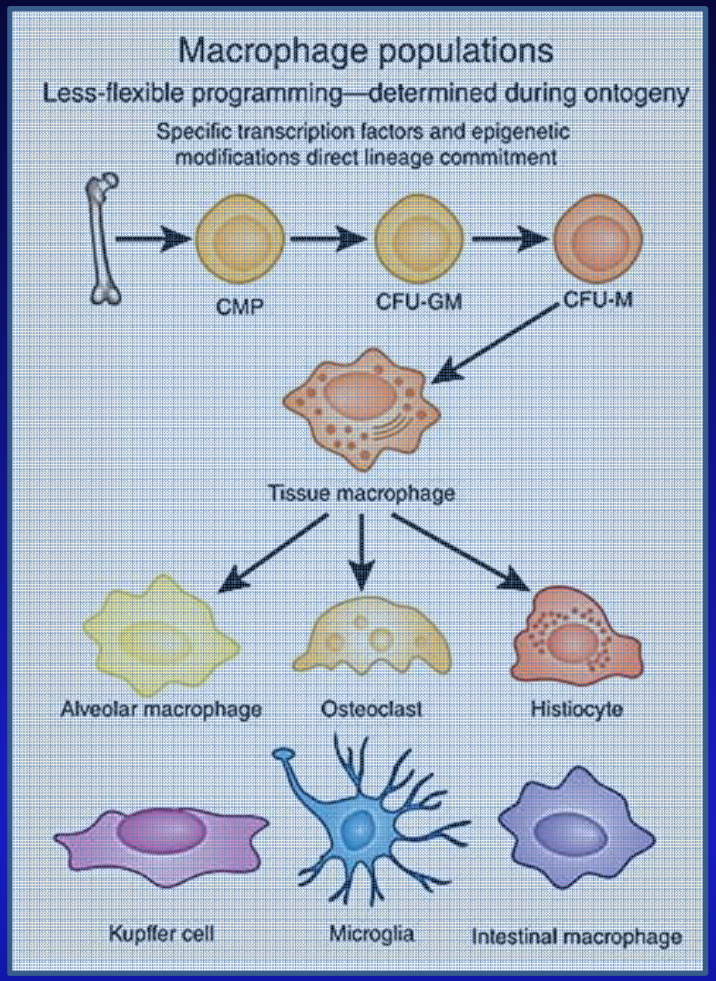
DCs take up antigens from infected or dying cells via macropinocytosis, phagocytosis, and endocytosis.

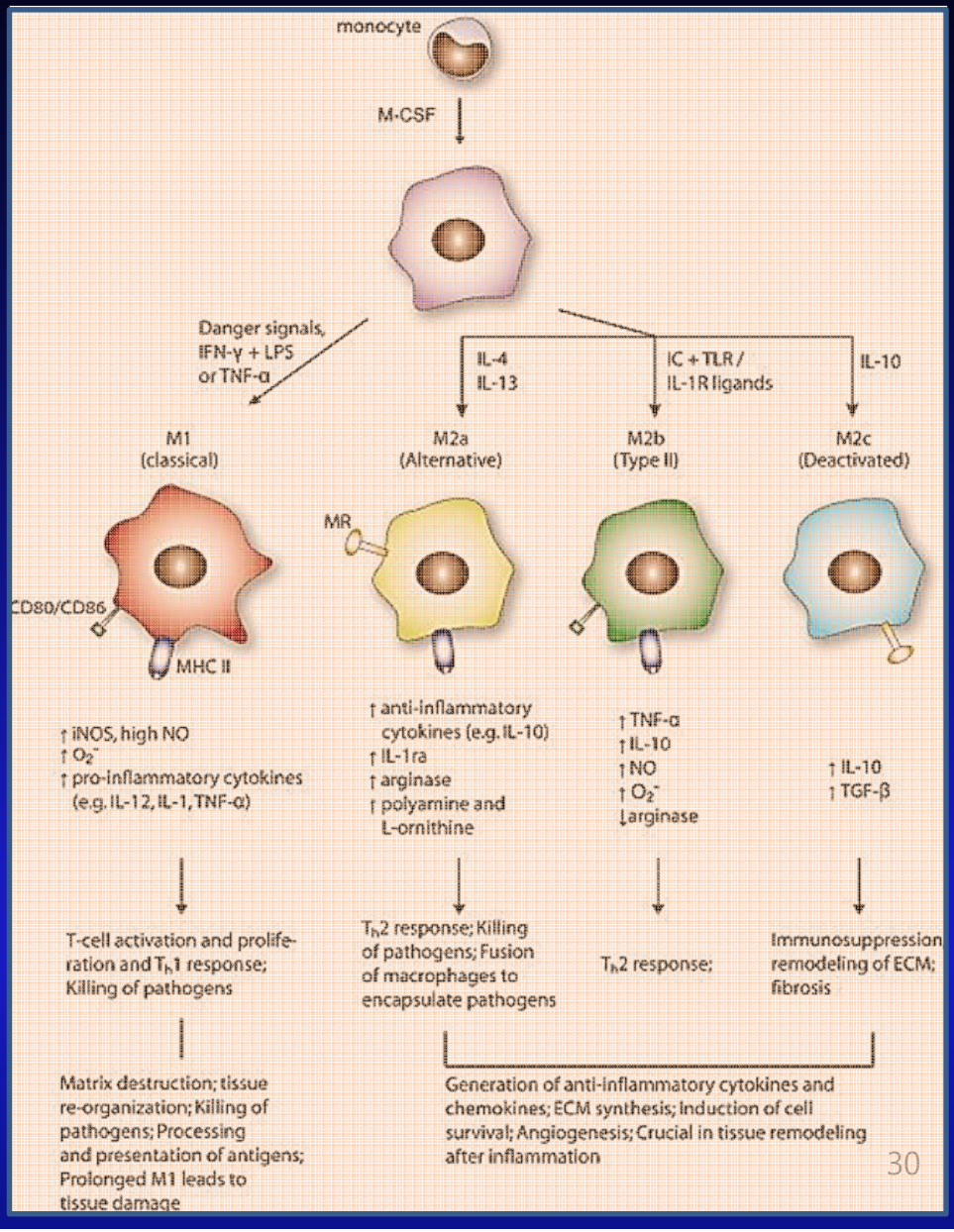
Immune inhibition is linked to the induction of regulatory DCs

Cytokines and Immune Diseases



Macrophage Populations and Functional Subsets





The phenotype and functions of different types of DCs and Mϕ

Phenotypic Attribute or Role	DCs	Mϕ
Experimental identification for human monocyte-derived cells	$CD14^{-}$, $CD1a^{+}$, $DC\text{-}SIGN^{+}$, $CD49d^{-}$, $CD49f^{-}$	$CD14^{+}$, $CD1a^{-}$, $DC\text{-}SIGN^{-}$, $CD49d^{+}$, $CD49f^{+}$
Sentinel in tissue	√	√
Phagocytic capacity	√	√
Antigen presentation	√	√
Production of complement proteins	√	√
Presence of proinflammatory and anti-inflammatory cell types	√	√
Naïve T-cell stimulation	√	X
Tolerogenesis	√	X
Innate cytotoxicity and antiviral capacity	X	√
Foreign body reaction	X	√

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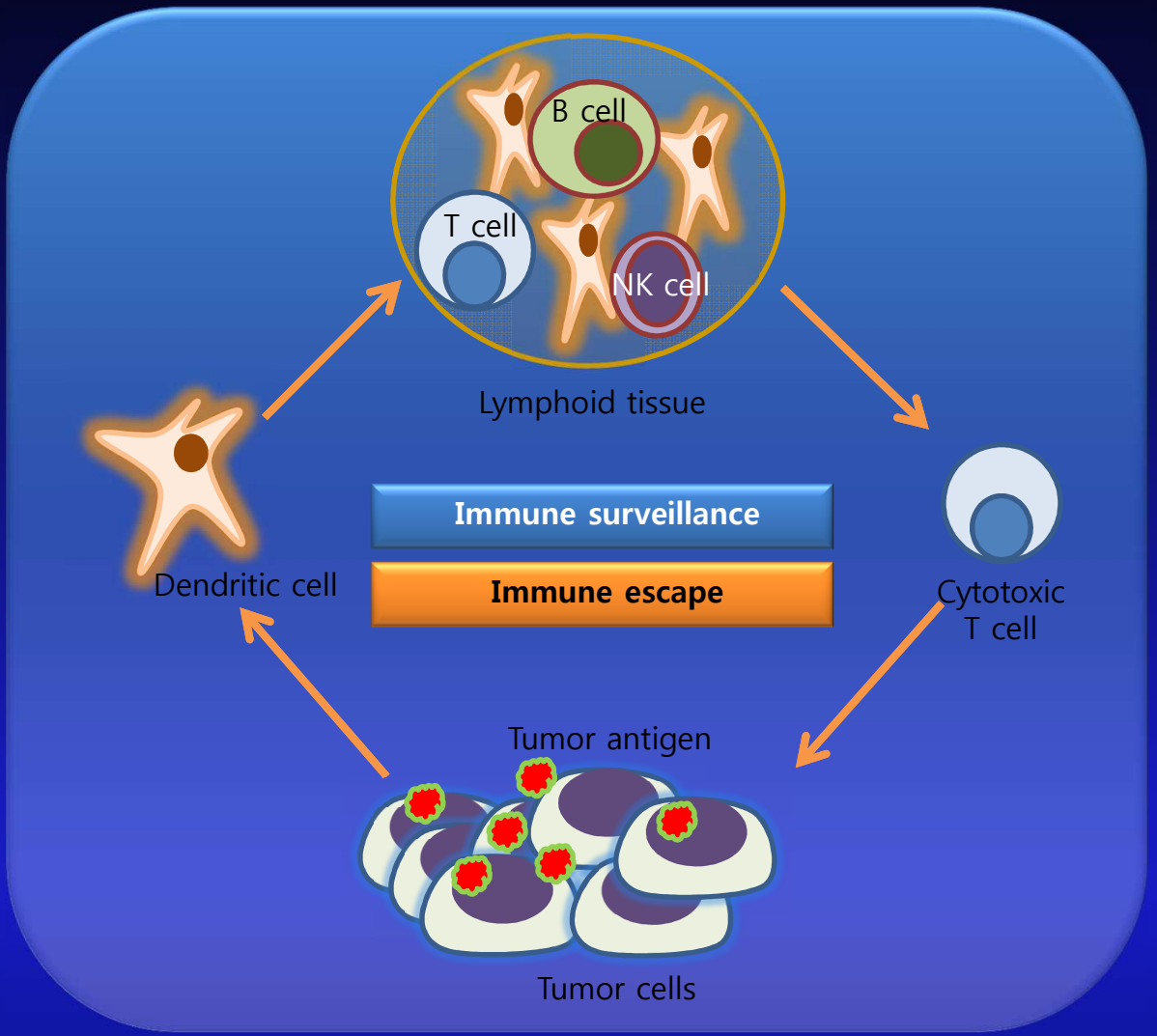
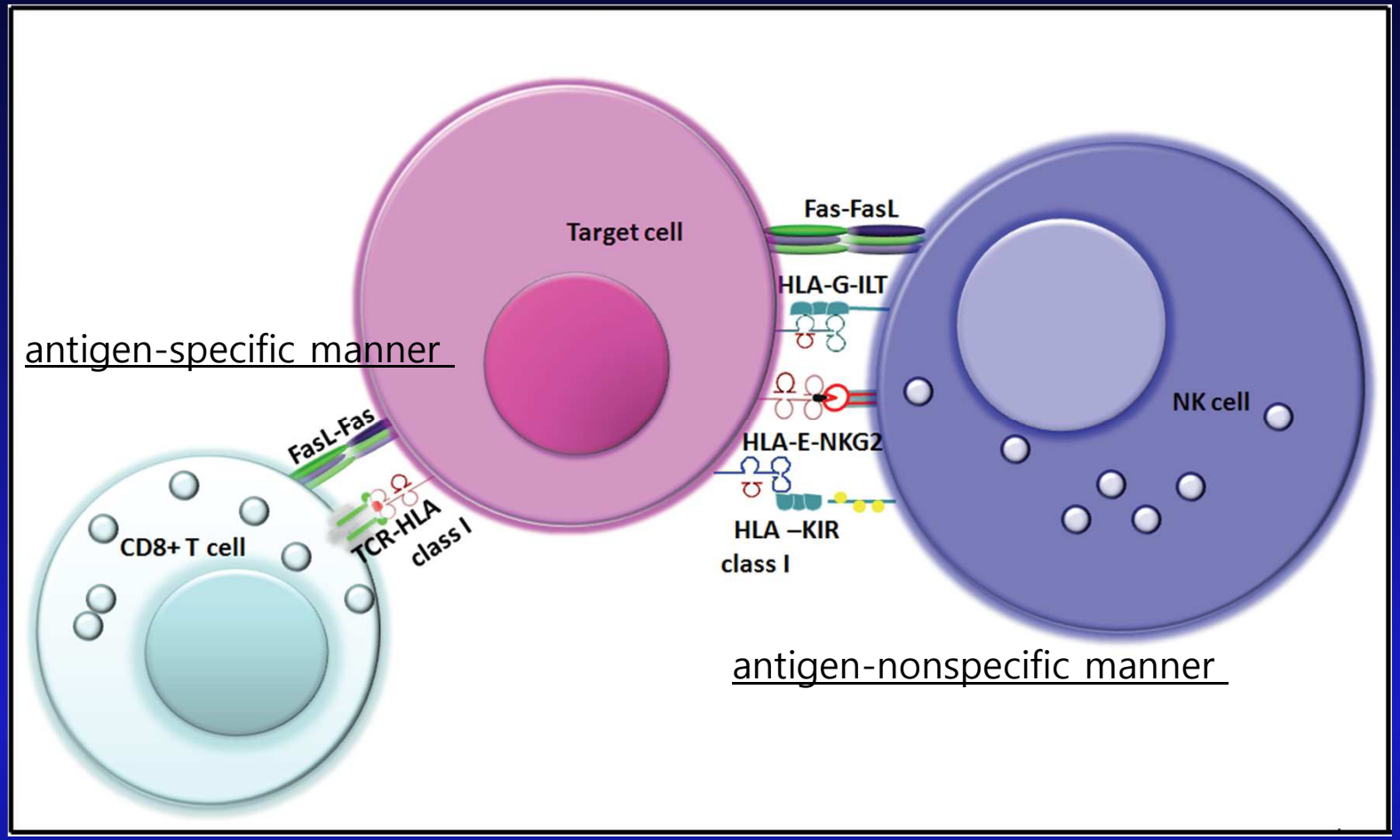
4. **Link to Adaptive Immunity against Self-modified Antigens**

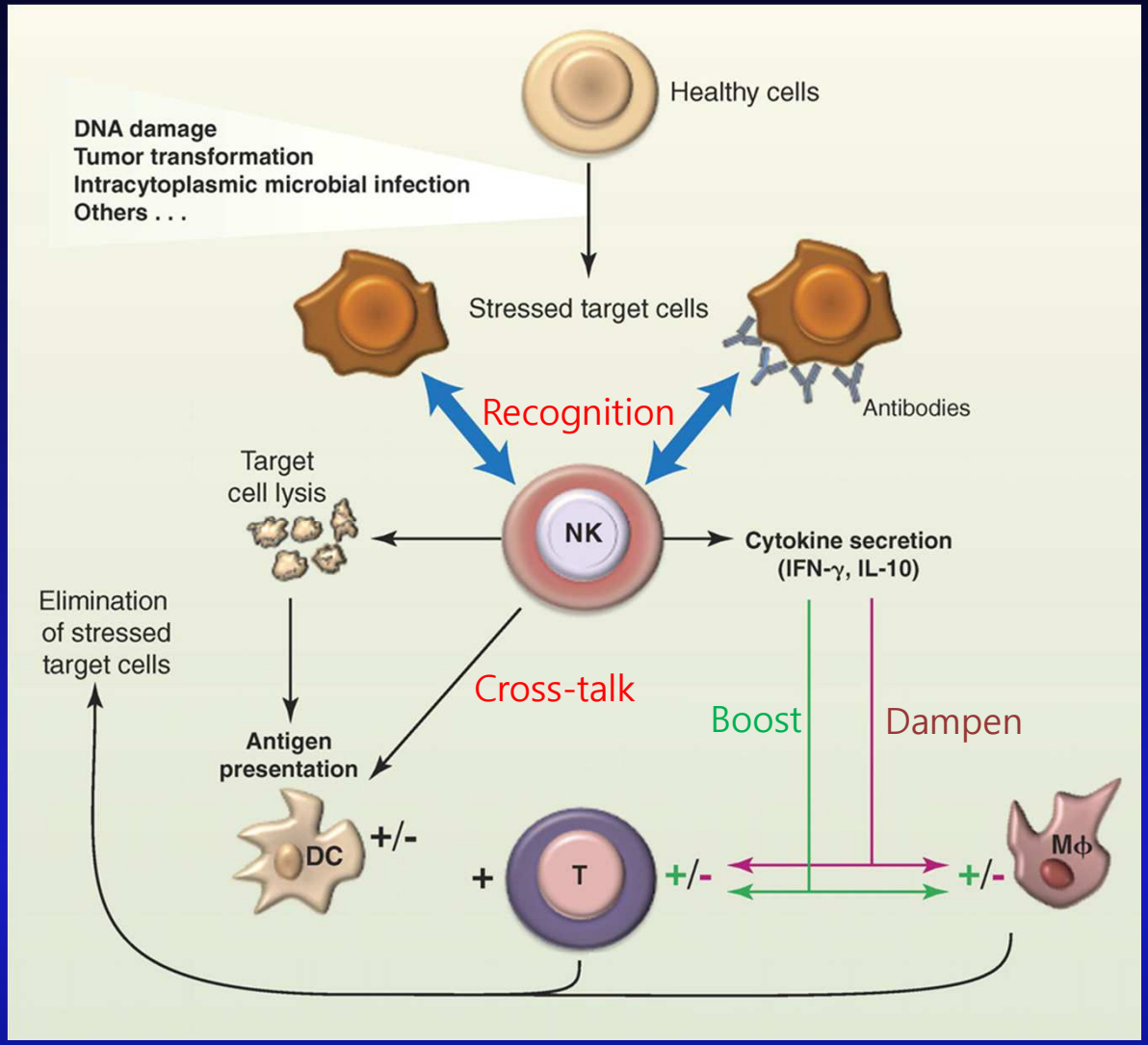
❖ Immune Response and Net-work to Tumor Cells



CD8+ T Cells and NK Cells Recognize the Target Cell through Different Receptors



The Biological Functions of NK Cells



Hallmarks of the Immune Infiltrates

	Pro-tumorigenic inflamation	Anticancer immunosurveillance
Cell types	M2 macrophages Myeloid-derived suppressor cells Neutrophils Foxp3$^+$ T reg, Th17 cells	Dendritic cells M1 macrophages Cytotoxic CD8$^+$ T cells with a memory effector phenotype
Cytokine profiles	Th2 Th17	Th1 CX3CL1 CXCL9, CXCL10
Distribution	Peritumoral	Intratumoral, close to cancer cells, as well as in the invasive front
Associated features	Stat3 phosphorylation	High endothelial venules
Functional impact	Negative prognostic impact	Positive prognostic and predictive impact

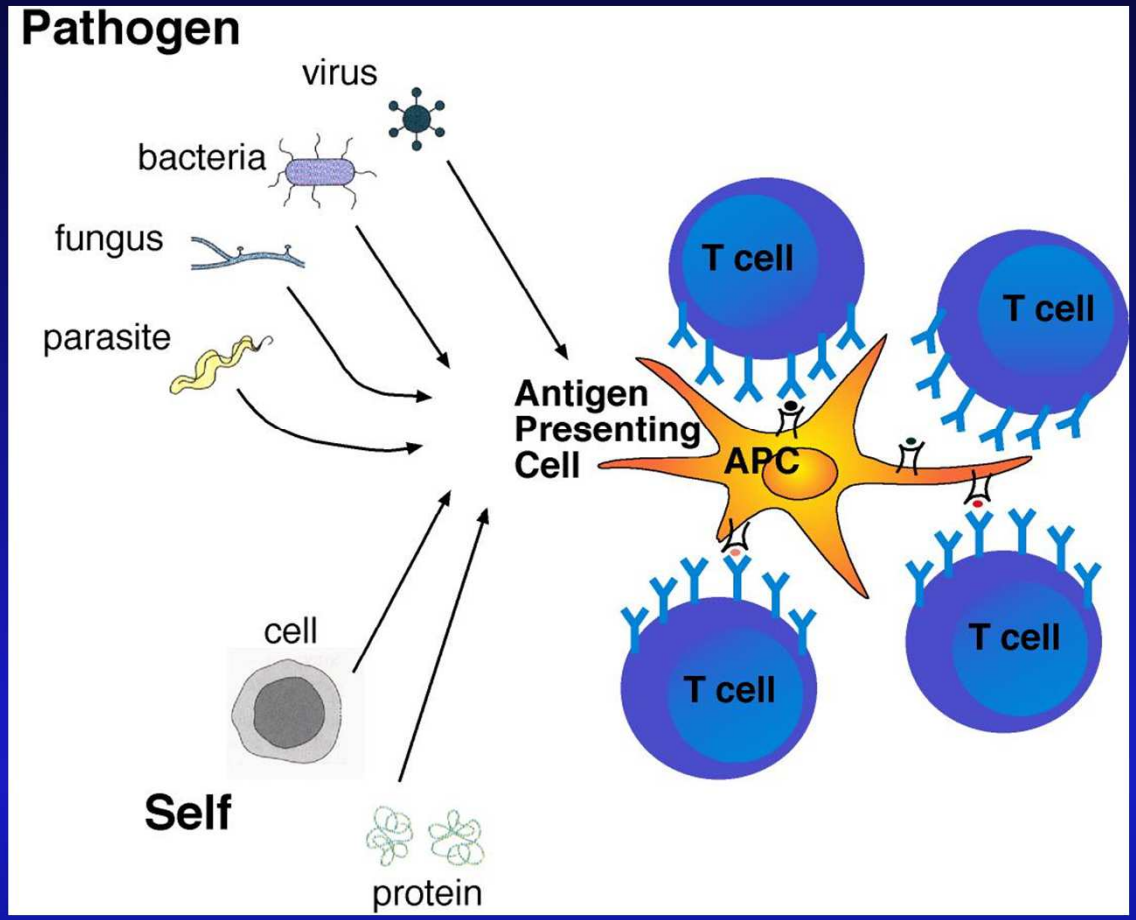
Cancer Research Reviews AACR

associated with inflammation vs. anticancer immunosurveillance

Dendritic Cells as Tumor-Associated Antigen (TAA)-Presenting Cells

Pathogen-associated molecular pattern

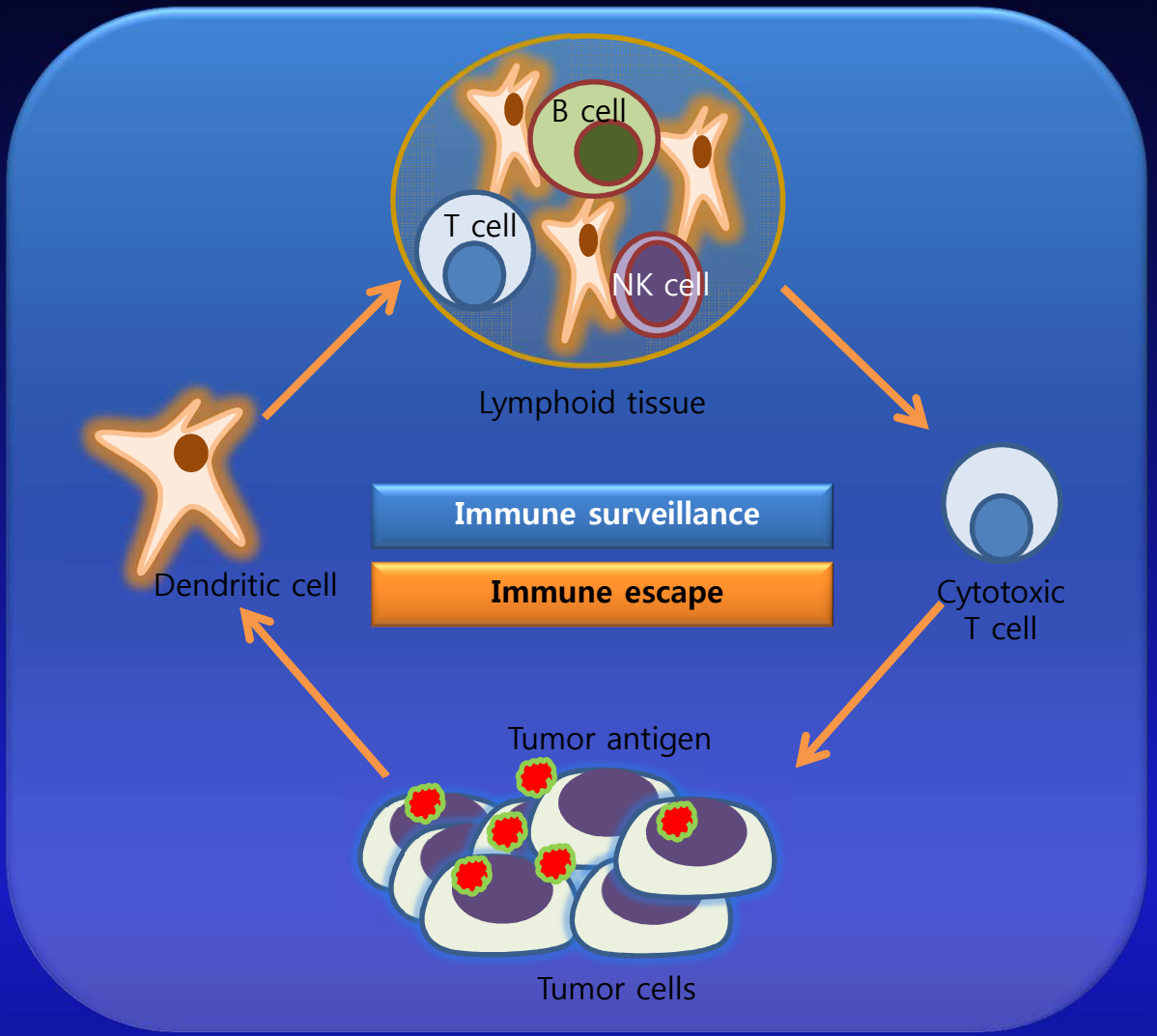
PAMPs



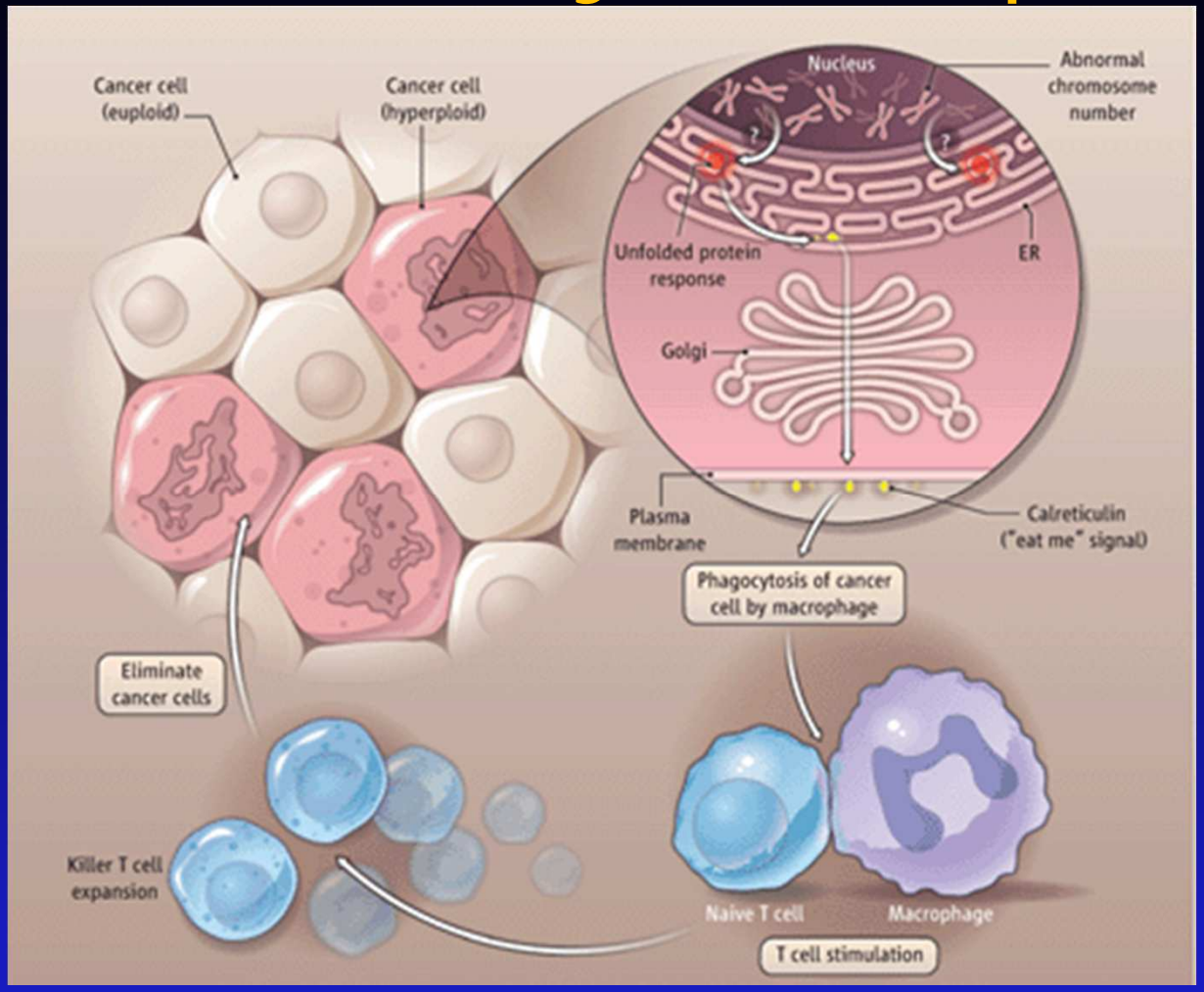
Damage-associated molecular pattern

DAMPs

❖ Immune Response and Net-work to Tumor Cells

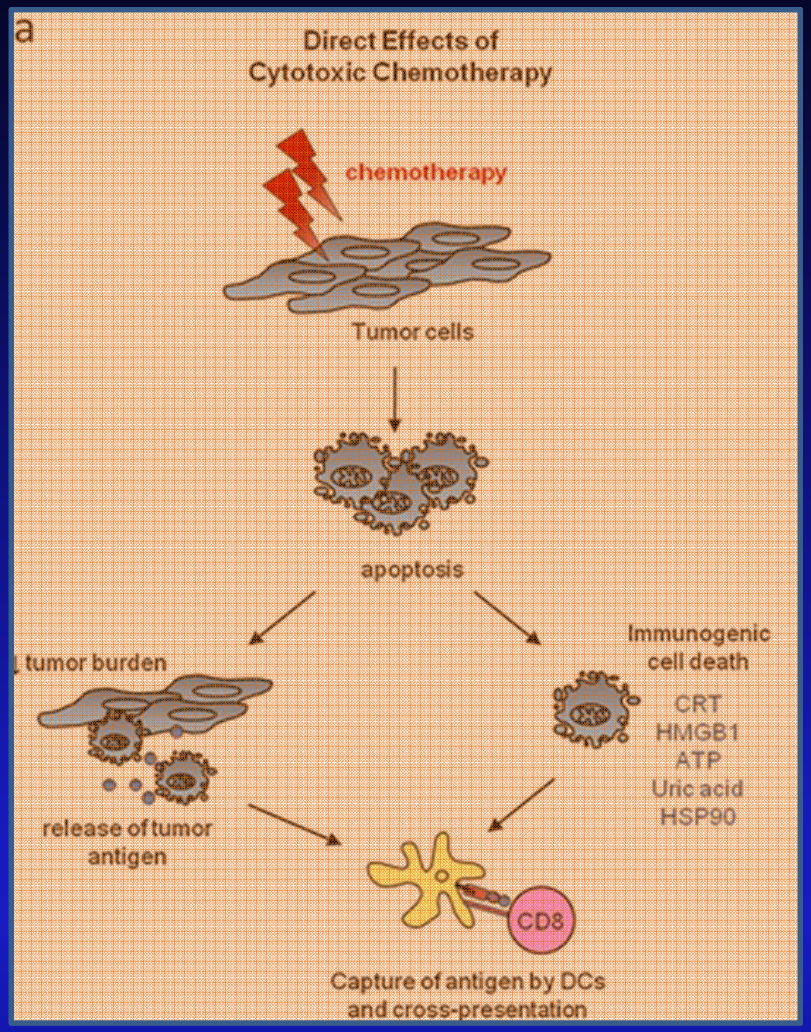


Cancer Cells Eliciting Immune Response



Order from chaos.
Hyperploidy in cancer cells activates the unfolded protein response in the endoplasmic reticulum (ER), which promotes the export of the ER-resident protein calreticulin to the cell surface where it elicits phagocytosis by macrophages and dendritic cells. These, in turn, present cancer cell antigens to T cells, driving their clonal expansion. The resulting killer T cells preferentially attack hyperploid cells, leading to attenuation or arrest of tumor growth.
CREDIT: Y. HAMMOND/*SCIENCE* 2012

Anti-tumor Chemotherapy and Immune Response



Chemotherapy induces apoptosis, leading to two effect on tumor

Cell Death

Cell Death Differ. 2012 Jan;19(1):107-20.

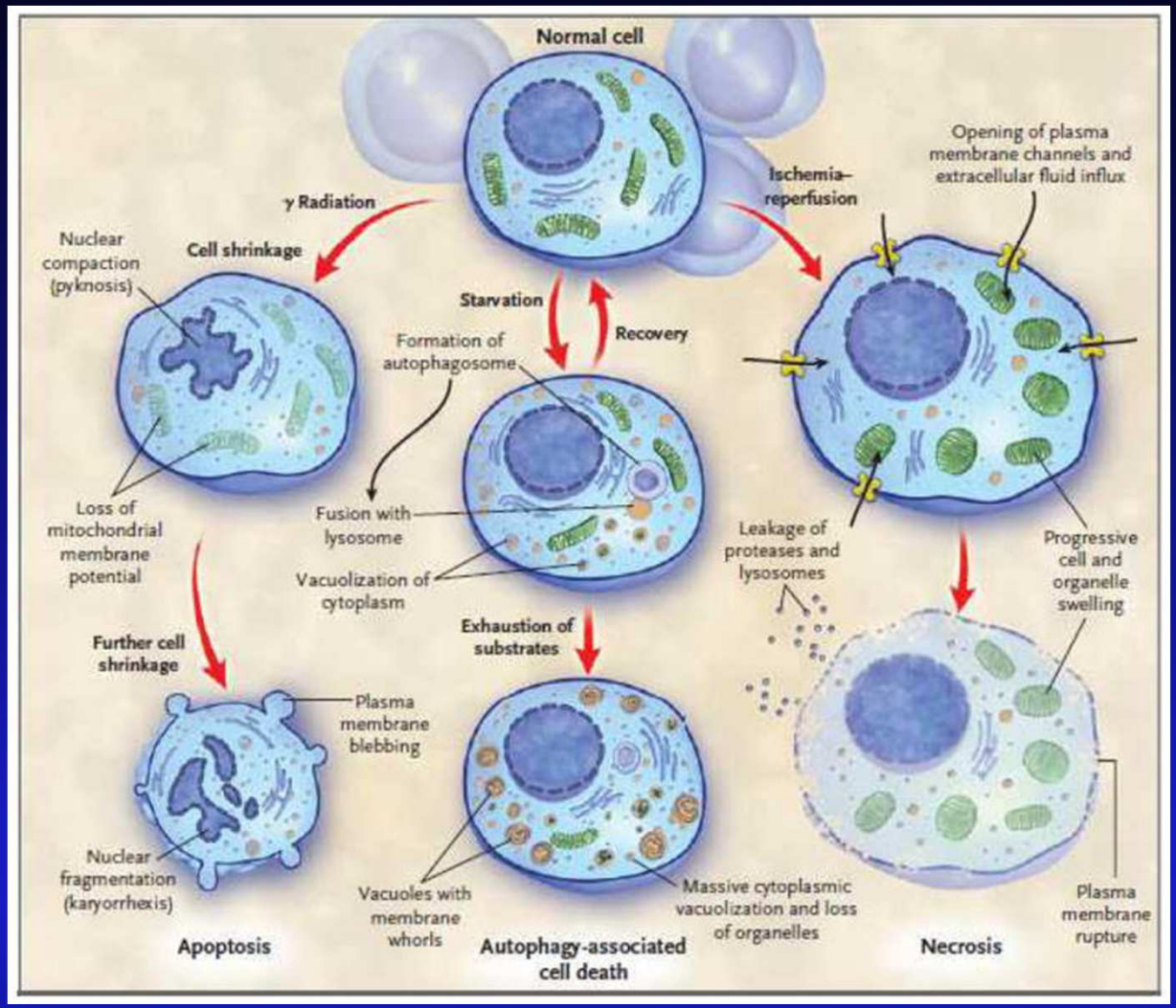
Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012.

INSERM U848, Apoptosis, Cancer and Immunity, Villejuif, France.

Abstract

In 2009, the Nomenclature Committee on Cell Death (NCCD) proposed a set of recommendations for the definition of distinct cell death morphologies and for the appropriate use of cell death-related terminology, including 'apoptosis', 'necrosis' and 'mitotic catastrophe'. In view of the substantial progress in the biochemical and genetic exploration of cell death, time has come to switch from morphological to molecular definitions of cell death modalities. Here we propose a functional classification of cell death subroutines that applies to both in vitro and in vivo settings and includes extrinsic apoptosis, caspase-dependent or -independent intrinsic apoptosis, regulated necrosis, autophagic cell death and mitotic catastrophe. Moreover, we discuss the utility of expressions indicating additional cell death modalities. On the basis of the new, revised NCCD classification, cell death subroutines are defined by a series of precise, measurable biochemical features.

Schematic Diagram Showing 3 Possible Pathways of Cell Death



Depending upon the injury and the type of cell

N Engl J Med. 2009 October 15; 361(16): 1570–1583

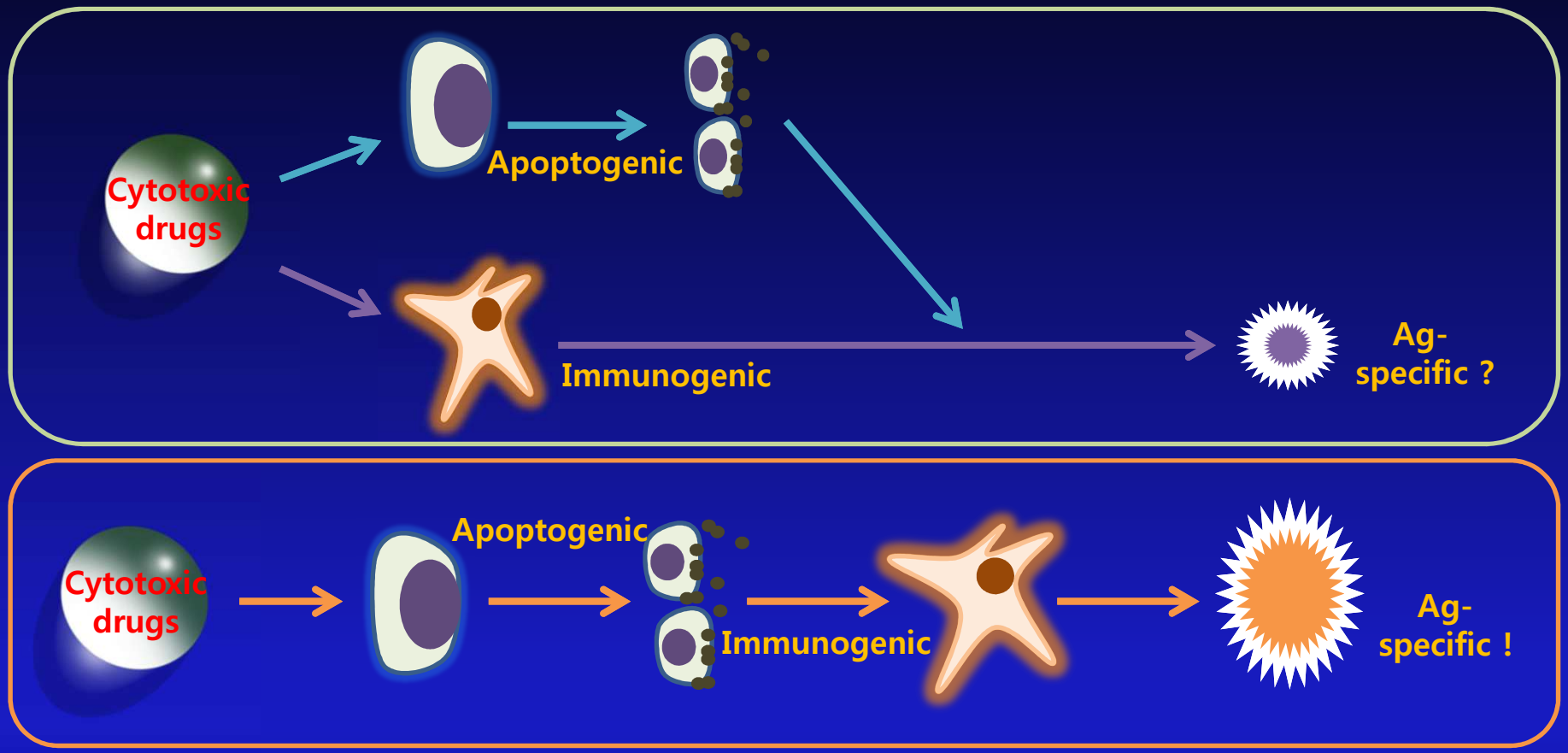
Features of Apoptosis and Necrosis

Morphology	Apoptosis	Necrosis
Cell	Shrinkage	Swelling
Mitochondria	Normal, although swelling possible late in the process	Marked swelling
Chromatin condensation	Present, classically with margination	Usually not prominent
Cell fragmentation	Membrane-enclosed apoptotic bodies	Cell rupture
Membrane blebbing	Present	Not characteristic
Membrane integrity	Intact in vivo; often lost at late time points (the latter especially in cell culture)	Defective at early stages
Tissue inflammation	Classically absent, although exceptions	Severe

Function	Apoptosis	Necrosis
Cellular ATP levels	Maintained	Markedly depleted
Production of ATP	Usually maintained but may decrease	Markedly decreased
Consumption of ATP	Decreased	Continues
MPTP opening	May occur late, but not a defining feature	An early defining event in the mitochondrial necrosis pathway
Loss of $\Delta\Psi_m$	May occur late, but not a defining feature	An early defining event in the mitochondrial necrosis pathway
Apoptogen release	Present due to Bax/Bak-dependent OMM permeabilization	Not classic, but may be present because of OMM rupture following MPTP opening

Circulation Research April 15, 2011 vol. 108 no. 8 1017-1036

Apoptosis and Immune Reaction
Vs.
Reaction to Immunogenic Apoptosis



Develop new anti-cancer drugs ?

No, Discover new function of clinically used drugs !

● Antitumor drugs – Immunogenicity test

DC-activating drugs | **Immunogenic death-inducing drugs**

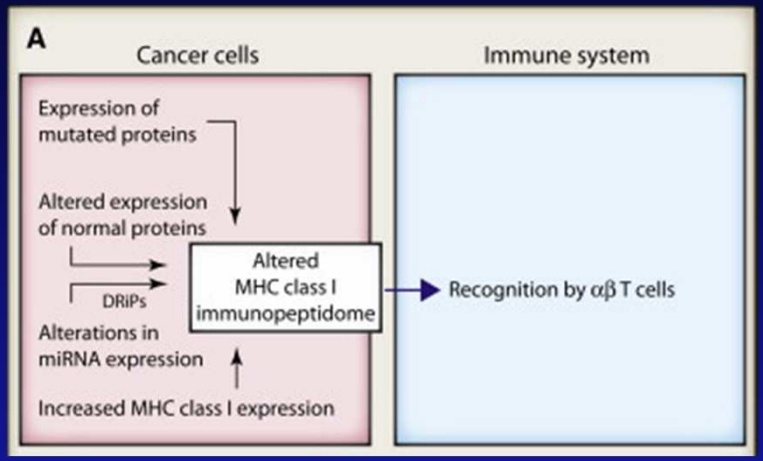
수지상세포활성 유도 항암제	면역원성 세포사멸유도 항암제
•vinblastine	•mitoxantrone
•paclitaxel	•daunoubicin
•docetaxel	•doxorubicin
•doxorubicin	•cyclophosphamide
•daunorubicin	•oxaliplatin
•mitoxantrone	•ionizing radiation
•mitomycin c	
•methotrexate	
•vincristine	

Off-Level Medication FDA-Approved

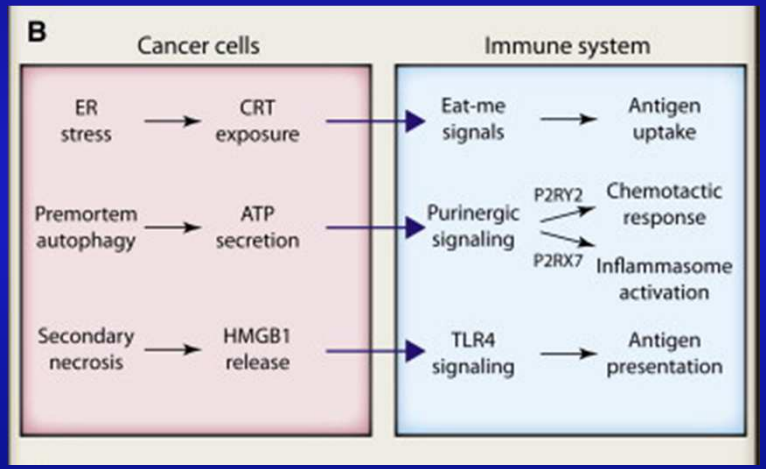
Location	Tumor type	Trials*	Phase	Notes
		Early clinical trials (phase I-II)		
Brain	Choroid plexus carcinoma	1	II	
	Embryonic brain tumors	1	II	
	Ependymoma	1	II	Often combined with platinum-containing regimens, EGFR inhibitors, etoposide or peptide-based vaccines.
	Glioblastoma	2	I-II	
	Medulloblastoma	2	II	
Colorectal tract	CRC	5	I-II	Combined with different immunostimulatory approaches.
Connective tissue	Osteosarcoma	1	II	Combined with sirolimus.
	Rhabdomyosarcoma	2	II	Combined with mAbs.
Epidermis	Melanoma	20	I-II	Often combined with fludarabine or immunostimulatory interventions.
Gastrointestinal system	Pancreatic cancer	5	II	Often combined with GM-CSF-based vaccines.
Hematological tumors	ATL	1	II	Combined with fludarabine.
	MDS	3	II	Often combined with ATG, fludarabine and stem cell transplantation.
	T-PLL	1	II	Combined with fludarabine and immunotherapy.
HNC	SCCHN	2	I	Combined with fludarabine and/or immunostimulatory interventions.
Kidney	Advanced or metastatic renal cancer	2	I-II	Combined with allogeneic HSCT, immunostimulatory interventions or everolimus.
Lung	Metastatic lung cancer	1	II	Combined with cancer vaccines.
	NSCLC	3	I-II	Combined with cancer vaccines.
	PPB	1	II	Combined with dactinomycin, doxorubicin, ifosfamide and vincristine.
	SCLC	1	II	In the context of the PCDE regimen.
Mesothelioma	-	2	I	Combined with immunotherapy.
Reproductive tract	Prostate cancer	5	I-II	Often combined with immunostimulatory approaches.
Thymus	Thymoma	2	I-II	Combined with belinostat, cetuximab, cisplatin or doxorubicin.
		Advanced clinical trials:phase III-IV		
Brain	AT/RT	1	III	Combined with cisplatin, etoposide, folinic acid, methotrexate and vincristine.
	Choroid plexus tumors	1	III	Combined with platinum-containing anticancer drugs, etoposide and vincristine.
	Ependymoma	2	III	Always combined with platinum-containing anticancer drugs and vincristine.
Lung	NSCLC	2	III	Always in combination with cancer vaccines.

Effects of Anticancer Agents on Tumor Antigenicity, Immunogenicity, and Susceptibility to Immune Attacks

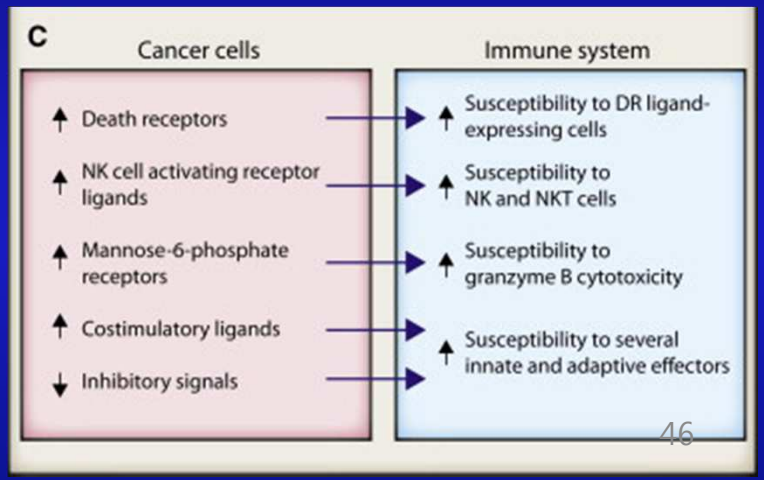
T cells



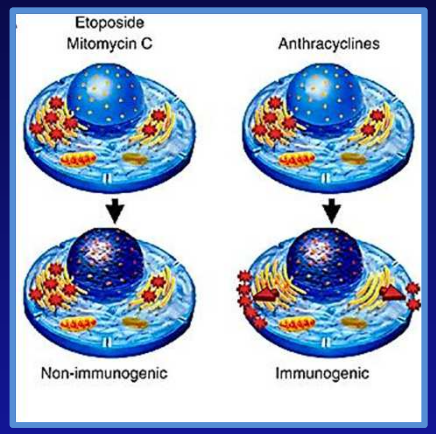
Dendritic cells

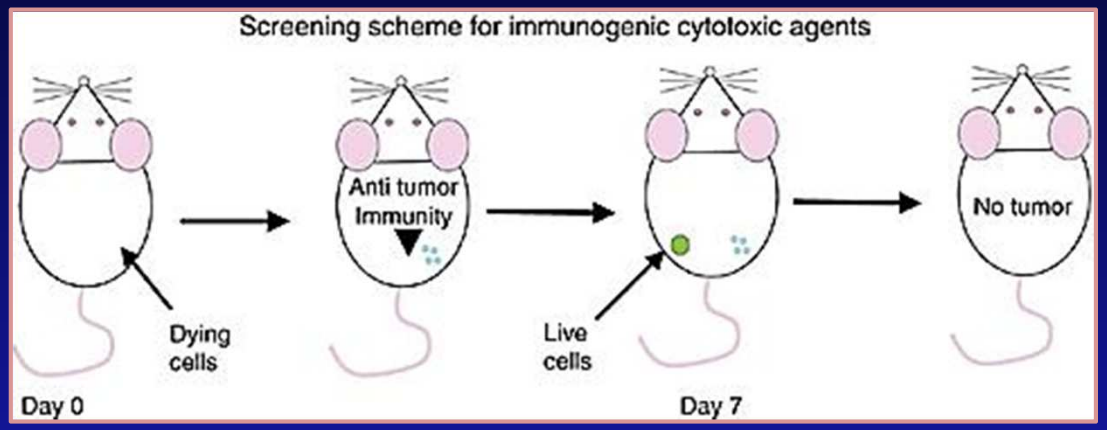


NK cells



Assay of immunogenic cell death of tumor cells





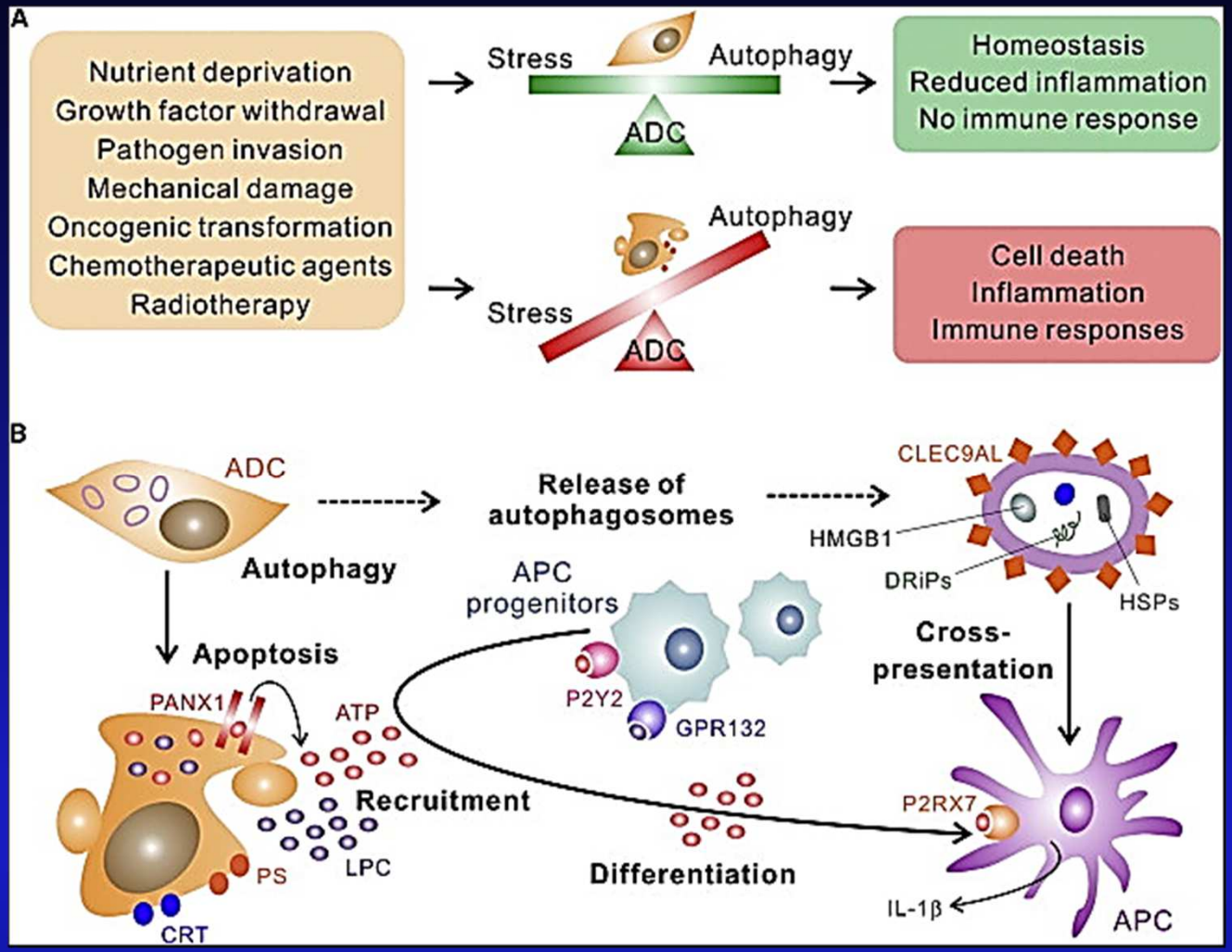
In vitro assay

- ✓Calreticulin exposure
- ✓ATP secretion
- ✓HMGB1 release

In vivo animal system

- ✓No growth of secondary injected tumor cells

Impact of Autophagy on Antigen Donor Cells (ADCs)



Immunity, Vol 39, Issue 2, 22 August 2013, Pages 211–227

Recent findings of immunogenic apoptosis

Immunity. 2013 Apr 18;38(4):729-41. doi: 10.1016/j.immuni.2013.03.003. Epub 2013 Apr 4.

Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells.

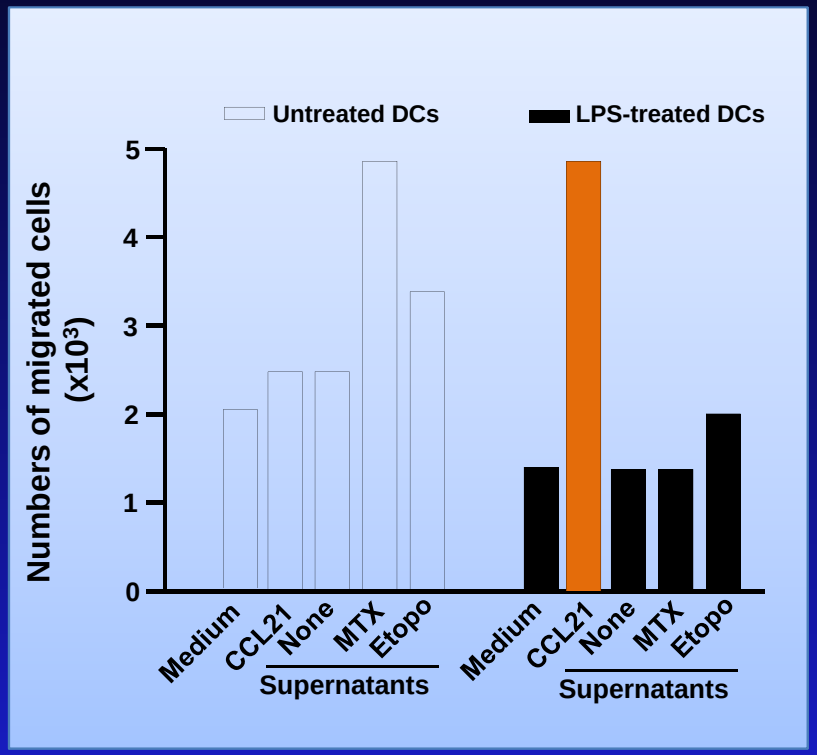
Ma Y, Adjemian S, Mattarollo SR, Yamazaki T, Aymeric L, Yang H, Portela Catani JP, Hannani D, Duret H, Steegh K, Martins I, Schlemmer F, Michaud M, Kepp O, Sukkurwala AQ, Menger L, Vacchelli E, Droin N, Galluzzi L, Krzysiek R, Gordon S, Taylor PR, Van Endert P, Solary E, Smyth MJ, Zitvogel L, Kroemer G.

Source

Institut National de la Santé et de la Recherche Médicale, U848, Villejuif 94805, France; Institut Gustave Roussy, Villejuif 94805, France; Université Paris Sud/Paris 11, Le Kremlin Bicêtre 94270, France.

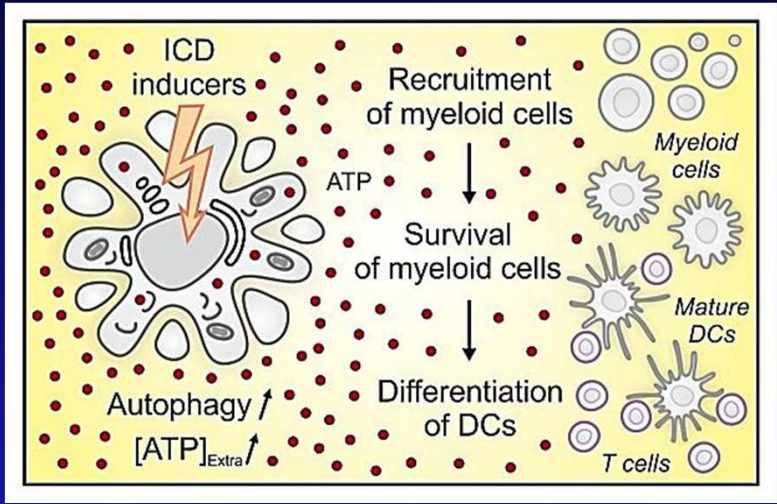
Abstract

The therapeutic efficacy of anthracyclines relies on antitumor immune responses elicited by dying cancer cells. How chemotherapy-induced cell death leads to efficient antigen presentation to T cells, however, remains a conundrum. We found that intratumoral CD11c(+)CD11b(+)Ly6C(hi) cells, which displayed some characteristics of inflammatory dendritic cells and included granulomonocytic precursors, were crucial for anthracycline-induced anticancer immune responses. ATP released by dying cancer cells recruited myeloid cells into tumors and stimulated the local differentiation of CD11c(+)CD11b(+)Ly6C(hi) cells. Such cells efficiently engulfed tumor antigens in situ and presented them to T lymphocytes, thus vaccinating mice, upon adoptive transfer, against a challenge with cancer cells. Manipulations preventing tumor infiltration by CD11c(+)CD11b(+)Ly6C(hi) cells, such as the local overexpression of ectonucleotidases, the blockade of purinergic receptors, or the neutralization of CD11b, abolished the immune system-dependent antitumor activity of anthracyclines. Our results identify a subset of tumor-infiltrating leukocytes as therapy-relevant antigen-presenting cells.

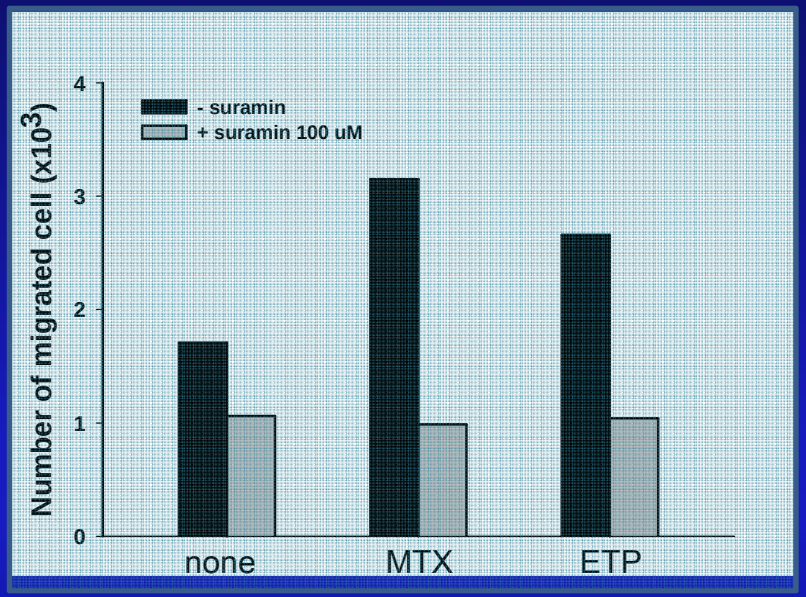


In Transwell Assay of DC migration

ATP-dependent Migration GMDCs to MTX-treated Cancer Cells

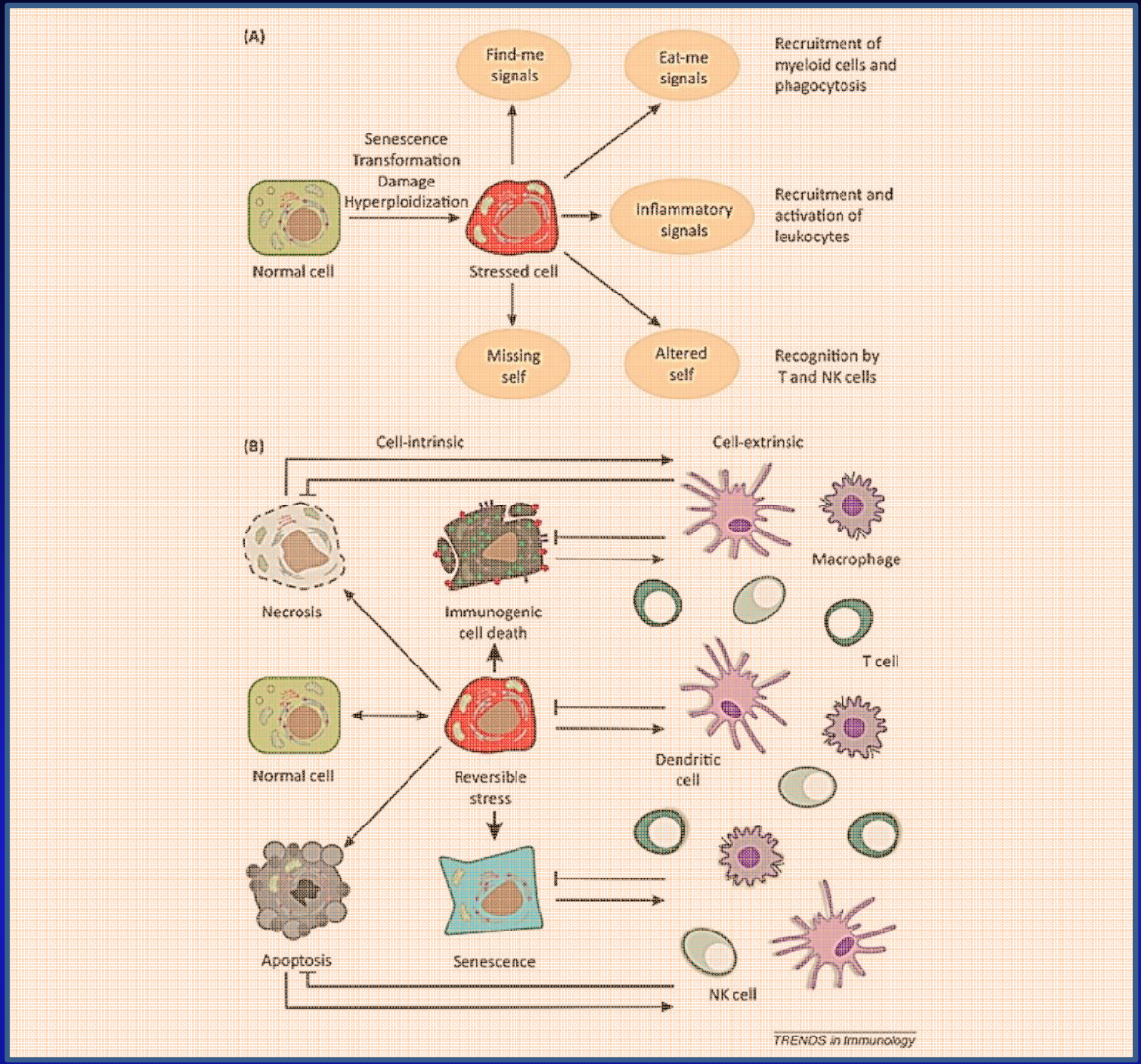


Facs	none	MTX	ETP
- suramin	1709	3149	2659
+ suramin	1063	988	1043

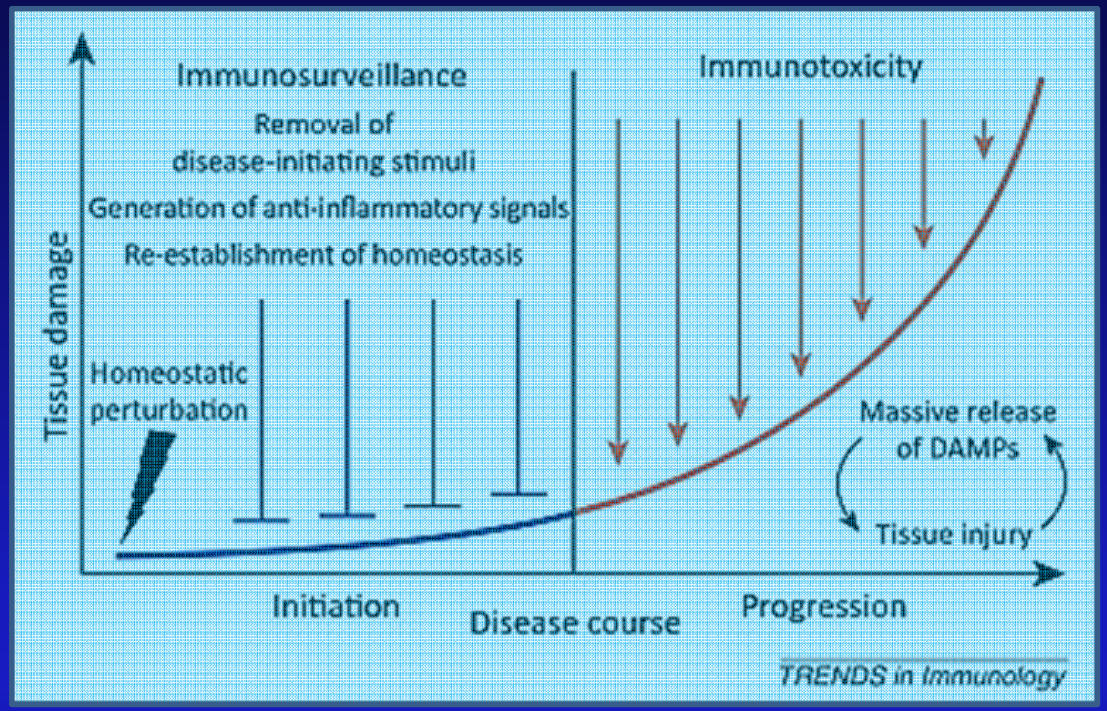


Oncoimmunology 2013 Jun 1;2(6):e24568.
ATP-dependent recruitment, survival and differentiation of dendritic cell precursors in the tumor bed after anticancer chemotherapy.
Ma et al., INSERM, U848; Villejuif, France.

General Organization of Cell-Intrinsic and Cell-Extrinsic Responses to Stress



Impact of the Immune System in the Loss of Tissue Homeostasis



Thank you for your attention

Welcome to Busan, Korea