4ο ΔΙΑΠΑΝΕΠΙΣΤΗΜΙΑΚΟ ΠΡΟΓΡΑΜΜΑ ΕΚΠΑΙΔΕΥΣΗΣ ΣΤΗ ΡΕΥΜΑΤΟΛΟΓΙΑ 2022-24

# *Βασικές αρχές μη ειδικής ανοσίας (κύτταρα & μηχανισμοί)*
# *Cellular mechanisms of innate immunity*


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## Immune response

*homeostasis*

PROTECTION vs TISSUE DAMAGE

Infection overcome vs Sepsis/Septic shock

# Δύο λειτουργικά σκέλη ανοσολογικής απόκρισης

- Μηχανισμοί φυσικής (innate)

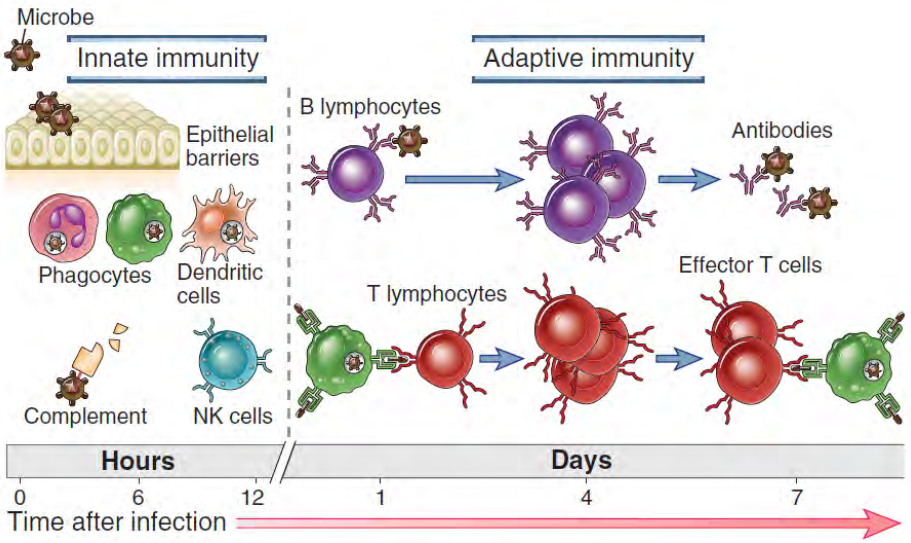
/μη ειδικής ανοσίας/έμφυτης

- Μηχανισμοί επίκτητης (adaptive)

/ειδικής ανοσίας/προσαρμοσμένης

**Φυσική και επίκτητη ανοσία**

Οι μηχανισμοί της φυσικής ανοσίας ενεργοποιούνται αρχικά. Οι μηχανισμοί της επίκτητης ανοσίας αναπτύσσονται αργότερα και απαιτούν την ενεργοποίηση των λεμφοκυττάρων



## Components (players) of innate immunity

- **physical and chemical barriers**

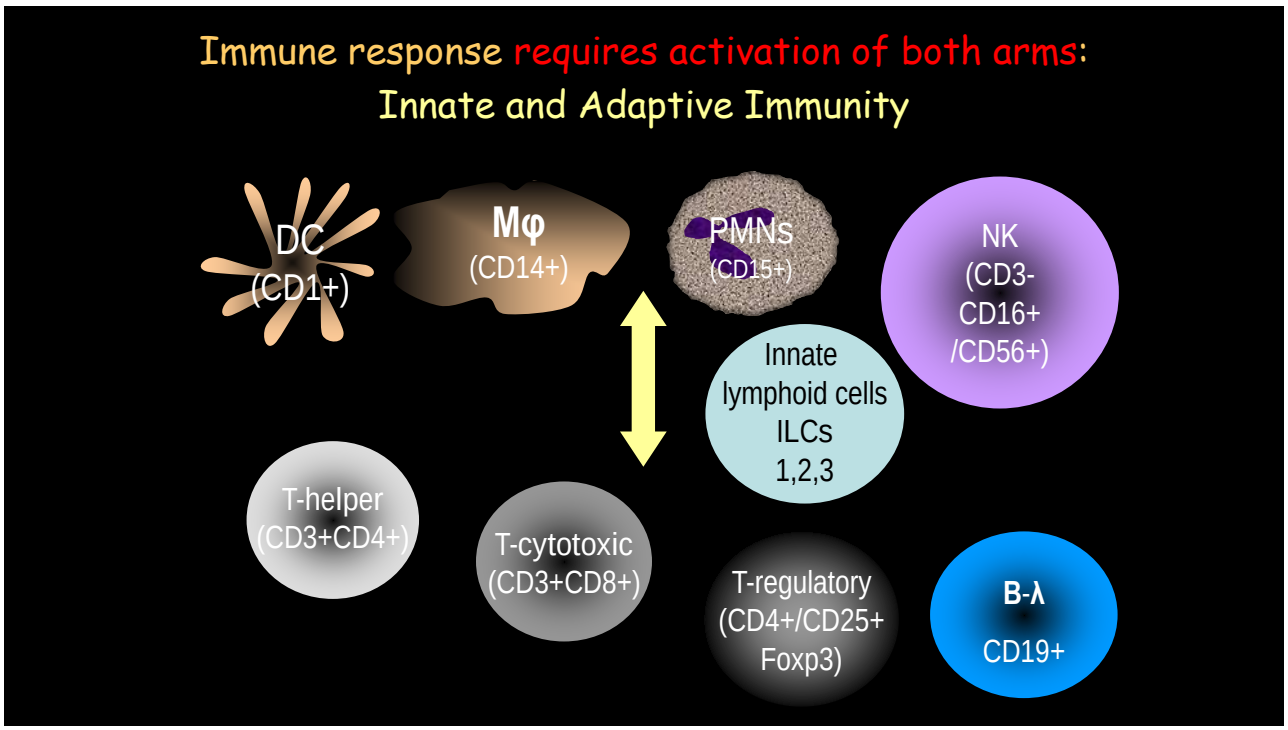
- epithelia (skin and the mucosal surfaces of the gastrointestinal, respiratory, and genitourinary tracts)
- antimicrobial substances produced at epithelial surfaces

- **cells**

neutrophils, macrophages, dendritic cells (DCs)
natural killer (NK) cells and other innate lymphoid cells (e.g. γδ-T)

- **blood proteins/mediators of inflammation (soluble factors)**

- complement and coagulation systems
- cytokines, interferons, chemokines



**What remains remarkable conserved during evolution in humans???**

**Innate immune system (σύστημα έμφυτης/φυσικής ανοσίας)**


## Charles Janeway,Jr (1989) Infectious-NonSelf Discrimination Model (INSD model)


•*Janeway CA Jr. Approaching the Asymptote? Evolution and Revolution in Immunology. Cold Spring Harbour Symposium Quant Biol 1989;54:1-13.*

•*Janeway CA Jr. The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol Today 1992;13:11-6.*

•*Medzhitov R, Janeway CA Jr. Innate immunity: impact on the adaptive immune response. Curr Opin Immunol 1997;9:4-9.*

•*Medzhitov R, Preston-Hurlburt P, Janeway CA Jr (1997) A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 388:394–397*

# PAMPs

## *(pathogen-associated molecular patterns)*

***evolutionary conserved structures/products,***

***no antigenic diversity,***

***essential for survival and virulence of the microbes***

***(infectious inflammation)***

- lipopolysaccharide (LPS)
- peptidoglycan (PG)
- lipoproteins
- lipoteichoic acid (LTA)
- mannanes
- unmethylated CpG DNA sequences
- dsRNA, ssRNA

| PAMP | pathogen | biologicaly active fragment |
|---|---|---|
| LPS | most Gram-negative bacteria | lipid A |
| lipoprotein, lipopeptide | eubacteria | di(three)Pam. Cys at N-term. |
| peptidoglycan | most bacteria | muropeptides |
| lipoteichoic acid | most Gram-positive bacteria | glycosidic link, glycolip. anch. |
| CpG | most pathogens | nonmethylated CpG ODN |
| lipoarabinomannan | mycobacteria | LAM, LM |
| N-formyl-Met | prokarionts | amino-terminal N-formyl-Met |
| mannanes and mannoproteins | yeasts | unknown |
| dsRNA | viruses | dsRNA |
| flagellin | most bacteria | N and C-terminal |
| zymosan | fungi | β-glucan |

# Polly Matzinger (1994) Danger Model


• *Matzinger P (1994) Tolerance, danger, and the extended family. Annu Rev Immunol 12:991–1045*

• *Matzinger P (1998) An innate sense of danger. Semin Immunol 10:399–415*


- Immune system recognizes tissue damage not only pathogens
- Activation by various alert/danger signals derived from damaged tissues

# DAMPs
## *(damage/danger-associated molecular patterns)*
## *sterile inflammation*

- DNA, chromatin, HMGB1
- ATP
- crystals (monosodium urate, cholesterol)
- oxidized LDL (oxLDL)
- stress-derived proteins (eg heat-shock proteins, HSPs)
- immunocomplexes
- ????????

**TABLE 1.2** Features of Innate and Adaptive Immunity

| | Innate | Adaptive |
|---|---|---|
| **Characteristics** | | |
| Specificity | For molecules shared by groups of related microbes and molecules produced by damaged host cells | For many different microbial and nonmicrobial antigens |
| Diversity | Low; recognition molecules encoded by inherited (germline) genes | Very high; many antigen receptors are generated by somatic recombination of gene segments in lymphocytes |
| Memory | Limited | Yes |
| Nonreactivity to self | Yes | Yes |
| **Components** | | |
| Cellular and chemical barriers | Skin, mucosal epithelia; antimicrobial molecules | Lymphocytes in epithelia; antibodies secreted at epithelial surfaces |
| Secreted proteins | Complement, various lectins | Antibodies |
| Cells | Phagocytes (macrophages, neutrophils), dendritic cells, natural killer cells, mast cells, innate lymphoid cells | Lymphocytes |

AK Abbas et al. Cellular and Molecular Immunology, 10th ed. 2021

**TABLE 1.2** Features of Innate and Adaptive Immunity

| | Innate | Adaptive |
|---|---|---|
| **Characteristics** | | |
| Specificity | For molecules shared by groups of related microbes and molecules produced by damaged host cells | For microbial and nonmicrobial antigens |
| Diversity | Limited; recognition molecules encoded by inherited (germline) genes | Very large; receptor genes are formed by somatic recombination of gene segments in lymphocytes |
| Memory | None or limited | Yes |
| Nonreactivity to self | Yes | Yes |
| **Components** | | |
| Cellular and chemical barriers | Skin, mucosal epithelia; antimicrobial molecules | Lymphocytes in epithelia; antibodies secreted at epithelial surfaces |
| Blood proteins | Complement, various lectins and agglutinins | Antibodies |
| Cells | Phagocytes (macrophages, neutrophils), dendritic cells, natural killer cells, mast cells, innate lymphoid cells | Lymphocytes |

AK Abbas et al. Cellular and Molecular Immunology, 9th ed. 2018

**Table 1–2.** Features of Innate and Adaptive Immunity

| | Innate | Adaptive |
|---|---|---|
| Characteristics | | |
| Specificity | For structures shared by groups of related microbes | For antigens of microbes and for nonmicrobial antigens |
| Diversity | Limited; germline-encoded | Very large; receptors are produced by somatic recombination of gene segments |
| Memory | None | Yes |
| Nonreactivity to self | Yes | Yes |
| Components | | |
| Physical and chemical barriers | Skin, mucosal epithelia; antimicrobial chemicals | Lymphocytes in epithelia; antibodies secreted at epithelial surfaces |
| Blood proteins | Complement | Antibodies |
| Cells | Phagocytes (macrophages, neutrophils), natural killer cells | Lymphocytes |

AK Abbas et al. Cellular and Molecular Immunology, 5th ed. 2007

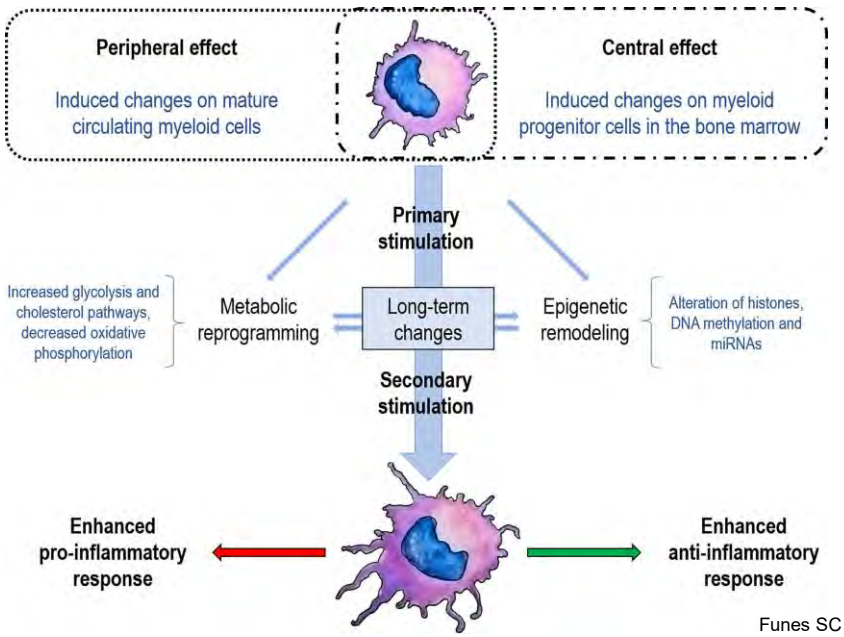
## Characteristics of Trained Innate Immunity (innate Immune memory)

Cell Host & Microbe
Perspective

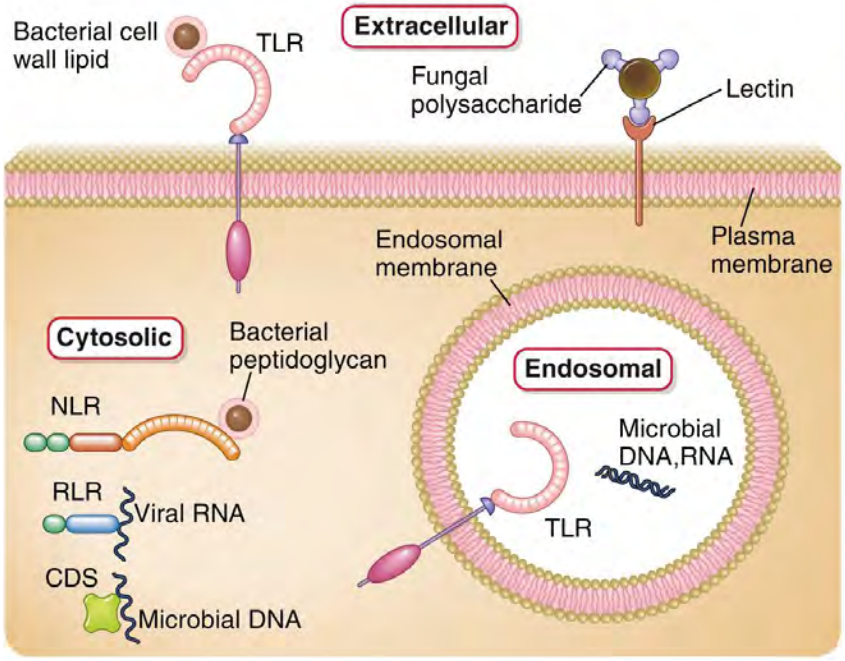
- Is induced **after a primary infection or vaccination**, and confers **protection against a secondary infection through mechanisms independent of T/B cell adaptive responses**.
- Increases resistance of the host to reinfection, but **is less specific than adaptive immunity** and thus may provide cross-protection to other infections.
- The mechanisms involve cells such as **macrophages** and **NK cells**, and entail improved pathogen recognition by PRRs and an **enhanced protective inflammatory response**.

Netea MG, Quintin J, van der Meer JW. Trained Immunity: A Memory for Innate Host Defense. Cell Host Microbe (2011)

## Trained immunity concepts




Funes SC et al. Front. Immunol 2022

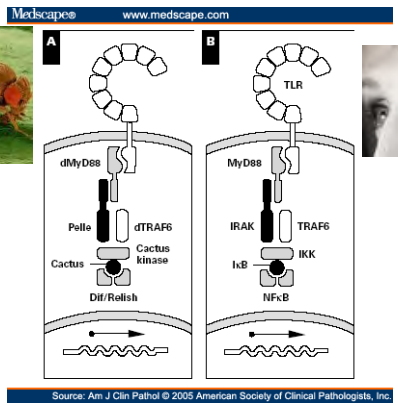


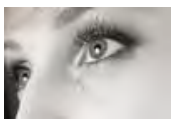

**PRRs (pattern recognition receptors): οι υποδοχείς των κυττάρων της φυσικής ανοσίας (Μφ, Dcs, PMNs)**

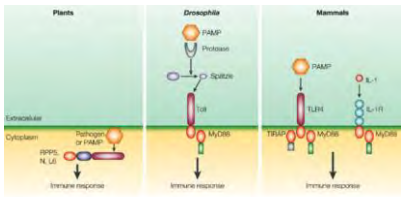
AK Abbas et al. Cellular and Molecular Immunology, 10th ed. 2021

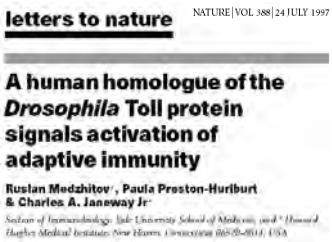
## Toll Like Receptors (TLRs):

- ✓ περιγράφηκαν για πρώτη φορά στη Δροσόφιλα (Nuesslein-Volhard, 1985)
- ✓ ρόλος στην ανοσία της Δροσόφιλας (Hoffmann 1996)
- ✓ ενεργοποίηση επίκτητης ανοσίας (Medzhitov & Janeway 1997)
- ✓ στον άνθρωπο 11 TLRs
- ✓ πυροδοτούν κοινές και ξεχωριστές σηματοδοτικές οδούς







letters to nature
NATURE | VOL 388 | 24 JULY 1997

A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity

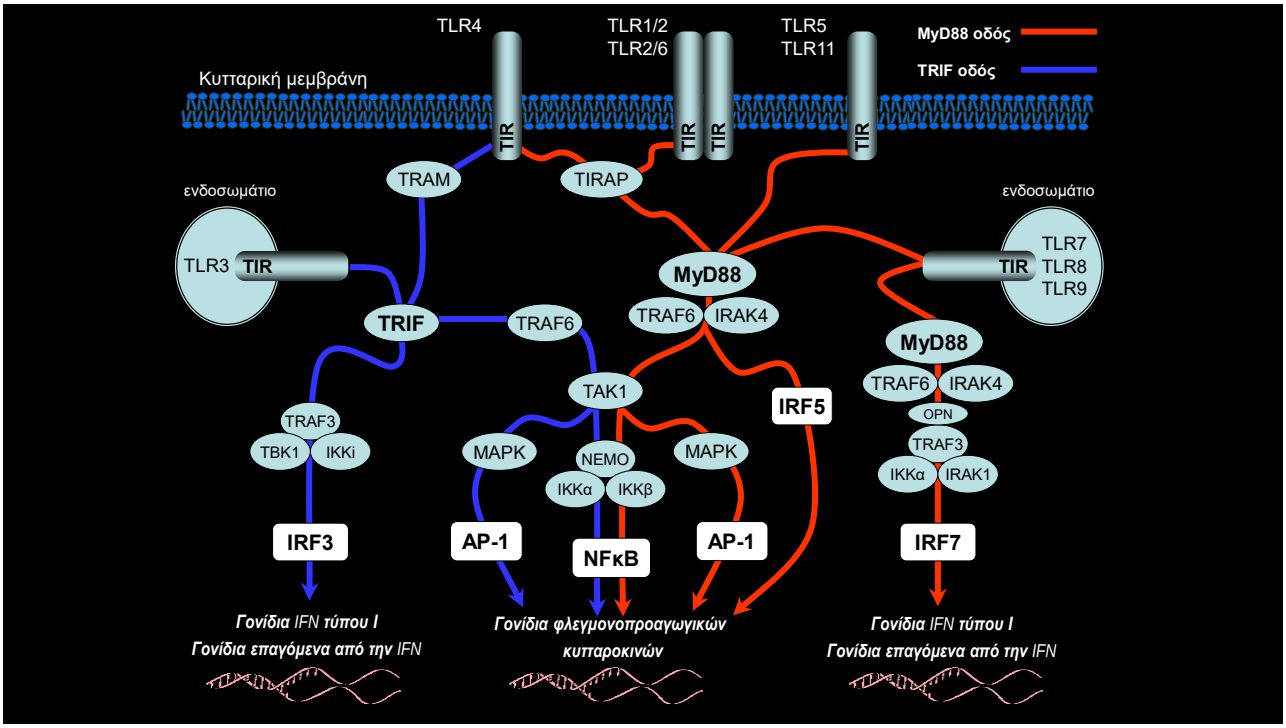
Ruslan Medzhitov*, Paula Preston-Hurlburt & Charles A. Janeway Jr*

*Section of Immunobiology, Yale University School of Medicine, and † Howard Hughes Medical Institute, New Haven, Connecticut 06510-8011, USA

## TLR κυτταρική κατανομή

| Υποδοχέας | Τύπος κυττάρου |
|---|---|
| TLR1 | παντού |
| TLR2 | ΔΚ, ΠΜΠ, και ΜΚ |
| TLR3 | ΔΚ και ΝΚ, επάγεται στα επιθηλιακά και ενδοθηλιακά |
| TLR4 | Μφ, ΠΜΠ, ΔΚ, ενδοθηλιακά |
| TLR5 | ΜΚ, ανώριμα ΔΚ, επιθηλιακά, ΝΚ και Τ |
| TLR6 | Υψηλή έκφραση στα Β, χαμηλή στα ΜΚ και ΝΚ |
| TLR7 | Β, πλασματοκυτταροειδή ΔΚ |
| TLR8 | ΜΚ, χαμηλή στα ΝΚ και Τ |
| TLR9 | πλασματοκυτταροειδή ΔΚ, Β, Μφ, ΠΜΠ, ΝΚ, και μικρογλοιακά |
| TLR10 | Β, πλασματοκυτταροειδή ΔΚ |
| TLR11 | ΜΚ/Μφ, ηπαρ, νεφρός, ουροθήλιο |

Οι περισσότεροι ιστοί εκφράζουν τουλάχιστον έναν TLR



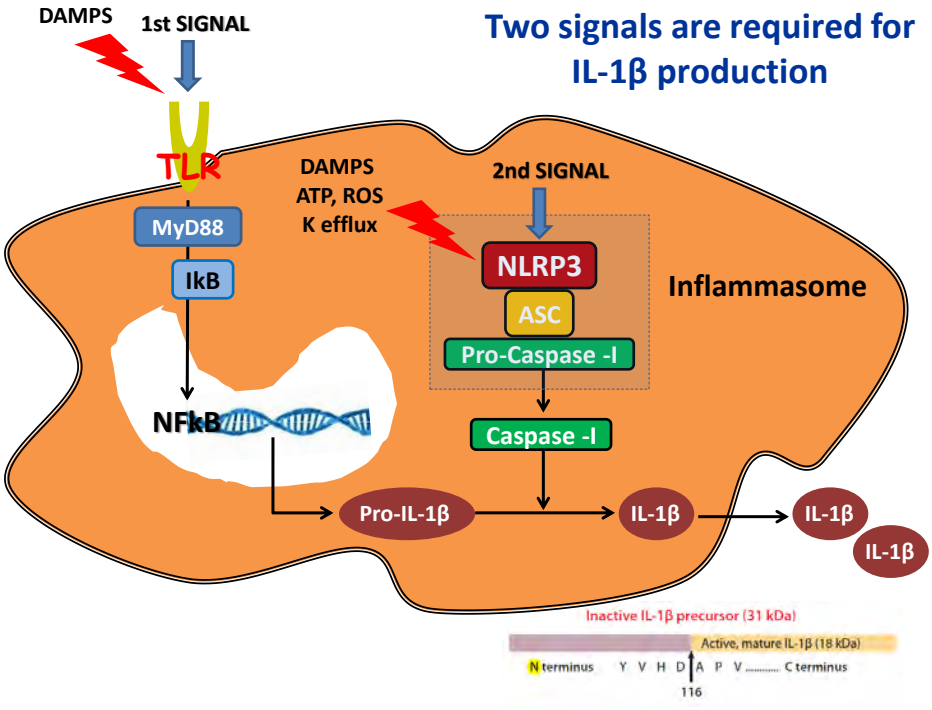
# Identification of the "inflammasome": a major breakthrough in the field of innate immunity & autoinflammation

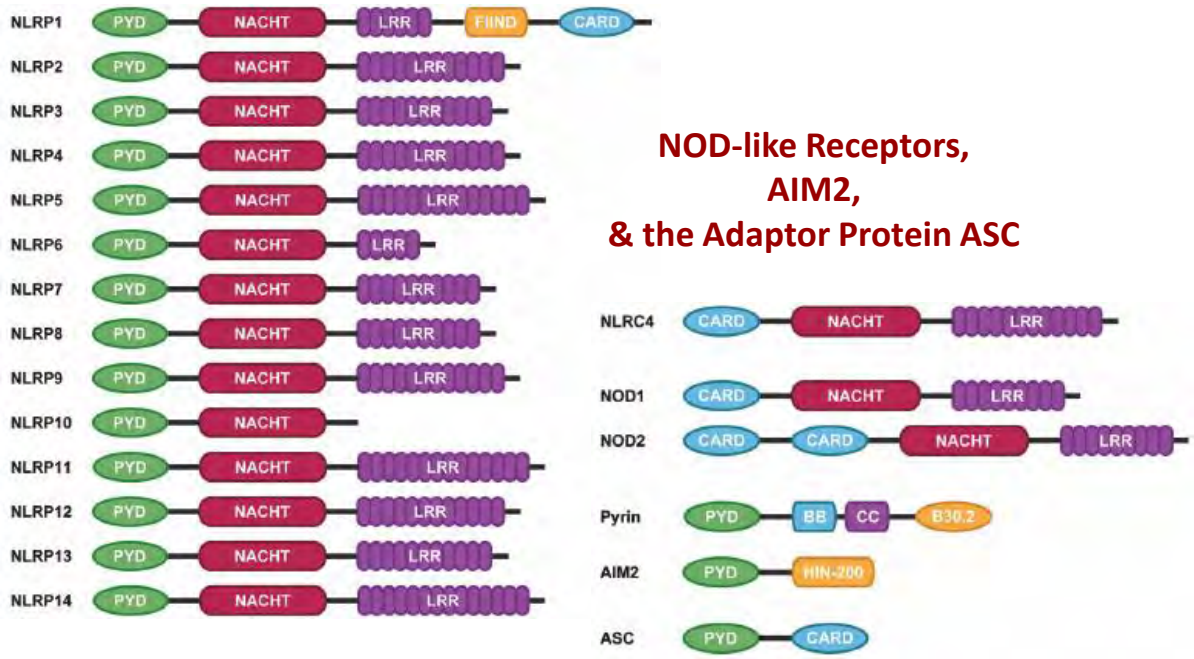
Molecular Cell, Vol. 10, 417–426, August, 2002, Copyright ©2002 by Cell Press

## The Inflammasome: A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL-β

**Fabio Martinon, Kimberly Burns, and Jürg Tschopp**[1]
Institute of Biochemistry
University of Lausanne
BIL Biomedical Research Center
Chemin des Boveresses 155
CH-1066 Epalinges
Switzerland

that they possess several distinct protein/protein interaction domains which are used to assemble large multicomponent complexes. Apaf-1, for example, contains an N-terminal CARD followed by a NBS/self-oligomerization domain and a C-terminal WD-40 repeat (Jaroszewski et al., 2000; Koonin and Aravind, 2000; van der Biezen and Jones, 1998) (Figure 1A). Via these domains, Apaf-1 assembles a complex (called the apoptosome)





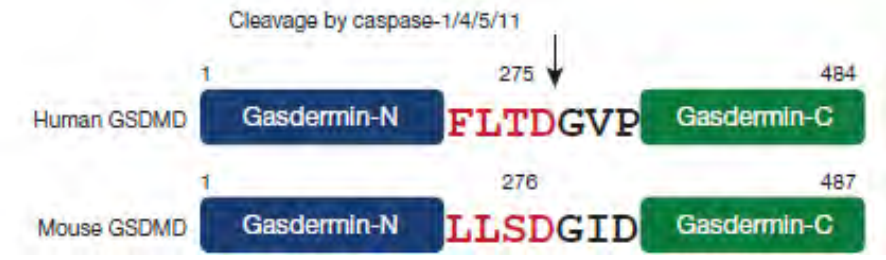

## NOD-like Receptors, AIM2, & the Adaptor Protein ASC

Platnich JM, Muruve DA. Arch Biochem Biophys. 2019

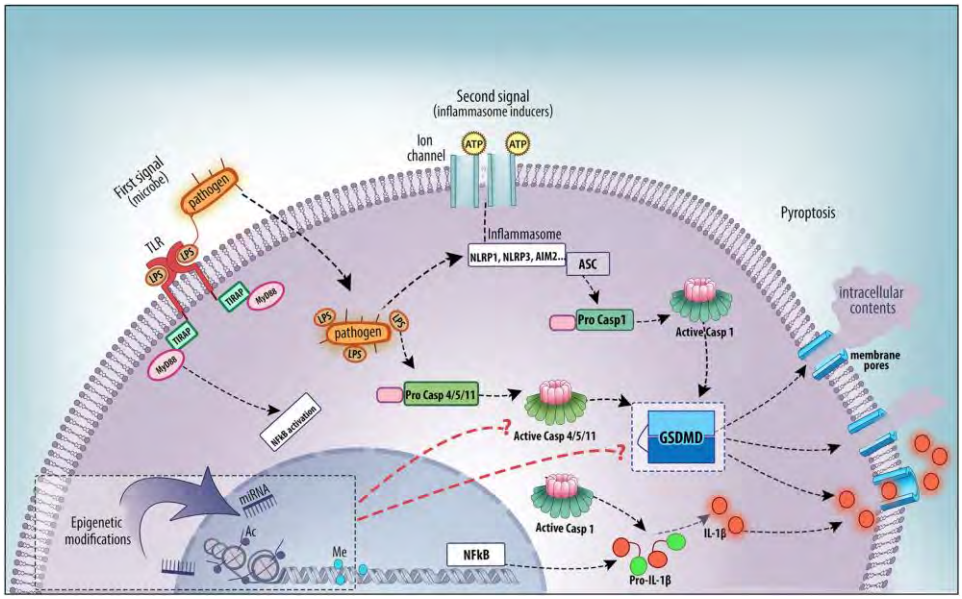
# Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death

Jianjin Shi[1,2]*, Yue Zhao[2]*, Kun Wang[2], Xuyan Shi[2], Yue Wang[2], Huanwei Huang[2], Yinghua Zhuang[2], Tao Cai[2], Fengchao Wang[2] & Feng Shao[2,3,4]

NATURE | VOL 526 | 29 OCTOBER 2015



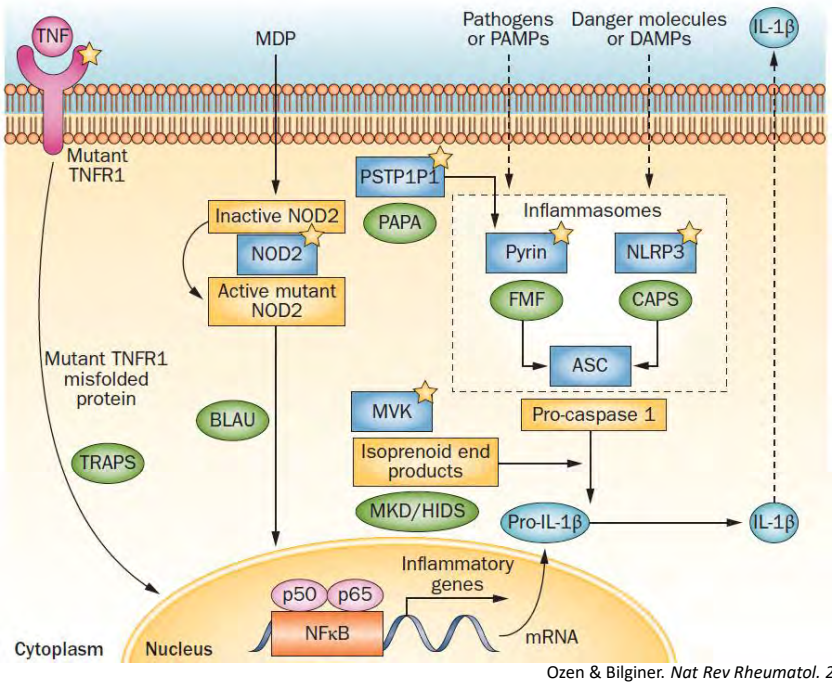
# Gasdermin D (GSDMD): A new player to the inflammasome




Ramos-Junior ES, Morandini AC. Biomed J. 2017

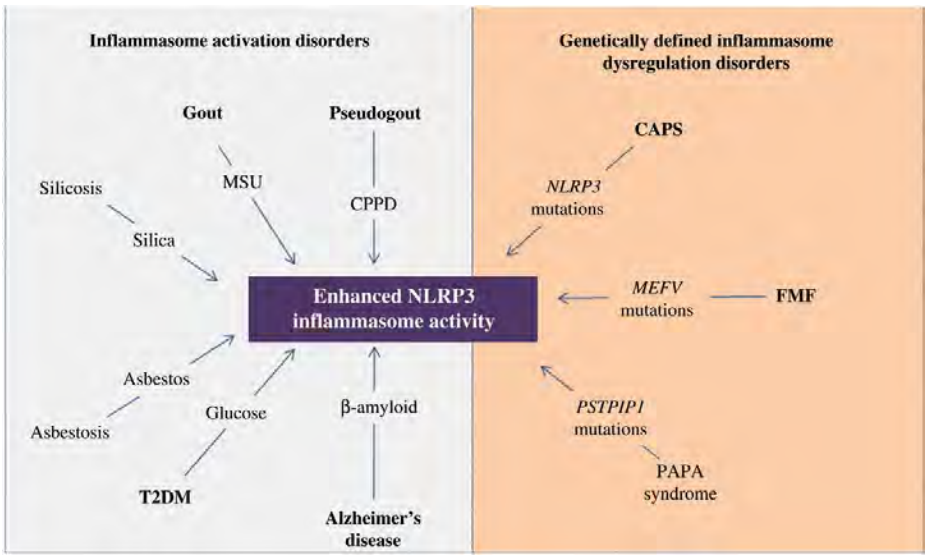
| Inflammasome | Structure | Stimulation signal | Downstream signal | Monogenic associated disorders |
|---|---|---|---|---|
| NLRP1 |  | Bacterial toxins, (i.e. *Bacillus anthracis toxin*), intracellular ATP depletion, muramyl dipeptide | Auto-cleavage of FIIND | NLRP1-associated autoinflammation with arthritis and dyskeratosis Crohn's disease (NAIAD); familial keratosis lichenoides chronica (FKLC); multiple self-healing palmoplantar carcinoma (MSCP) |
| NLRP3 |  | Extracellular ATP, Pore forming toxins (i.e. Nigericin), Crystalline, Particulate structures (silica, alum, asbestos, amyloid-β) | ROS, K+ efflux, intracellular $Ca^{2+}$, cAMP, phagosomal rupture | Cryopyrin-associated periodic syndromes (CAPS); familial cold autoinflammatory syndrome (FACS); Muckle-Wells syndrome (MWS); neonatal-onset multisystem inflammatory disease (NOMID/CINCA) |
| NLRC4 |  | Cytosolic flagellin (i.e. *Salmonella typhimurium*), Type III secretion system components (i.e. *Escherichia coli*) | Phosphorylation (Pkc δ kinase) | Syndrome of enterocolitis and autoinflammation associated with mutation in NLRC4 (SCAN4); macrophage activation syndrome (MAS) |
| AIM2 |  | Cytosolic dsDNA from DNA viruses or cytosolic bacteria (i.e. papillomavirus, *Mycobacterium tuberculosis*) | Direct recognition of dsDNA, Pyroptosis | |
| Pyrin |  | RhoA-GTPase inactivation (i.e. *Bordetella pertussis* (pertussis toxin), *Burkholderia cenocepacia* (TecA), *Clostridium botulinum* (C3 toxin), *Clostridium difficile* (TcdB), *Histophilus somni* (IbpA), *Vibrio parahaemolyticus* (VopS), *Yersinia pestis* (Yops)) | PKN1/2 inactivation, reduced pyrin 14-3-3 interaction | Familial Mediterranean fever (FMF); pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND); mevalonate kinase deficiency (MKD)/ hyperimmunoglobulinemia D syndrome (HIDS); pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome; WDR1 deficiency/PFIT |

Schnappauf O, Chae JJ, Kastner DL and Aksentijevich I. Front. Immunol. 2019




Ozen & Bilginer. *Nat Rev Rheumatol. 2014*

## Disorders that are associated with dysregulation of NLRP3 inflammasome and increased production of IL-1β




***Mitroulis I, Skendros P, Ritis K. Eur J Intern Med. 2010***

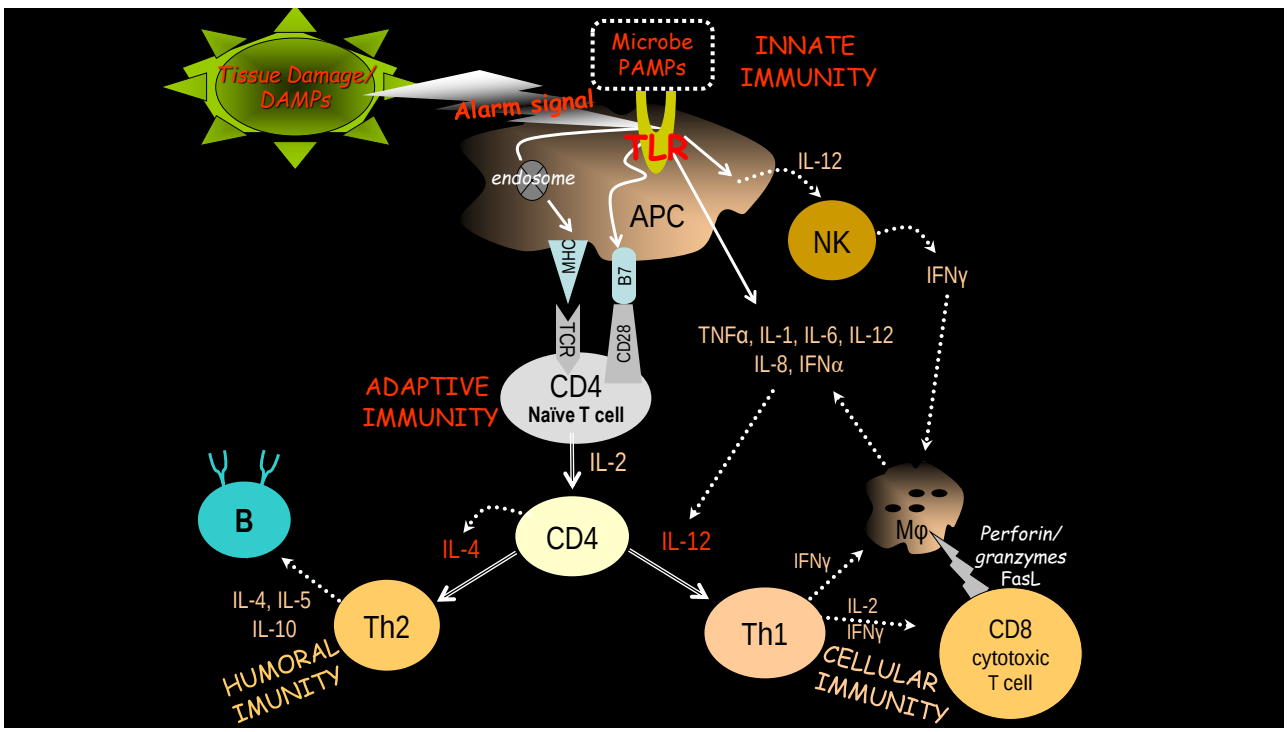
## Innate immunity:

Influence the **nature and type** (Th1,Th2) of adaptive immune response

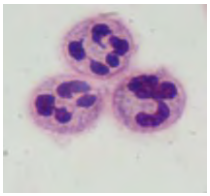
# Toll-like receptors control activation of adaptive immune responses

Markus Schnare[1,2,*] Gregory M. Barton[1,2,*], Agnieszka Czopik Holt[1], Kiyoshi Takeda[3], Shizuo Akira[3] and Ruslan Medzhitov[1,2]

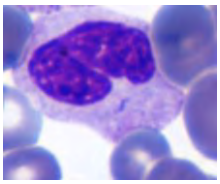
**Nature Immunology 2:947 (2001)**



# Cells of innate immuntiy

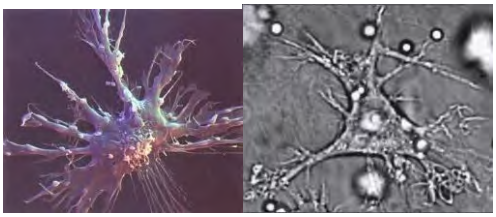


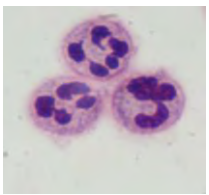
Neutrophils



Monocytes/macrophages

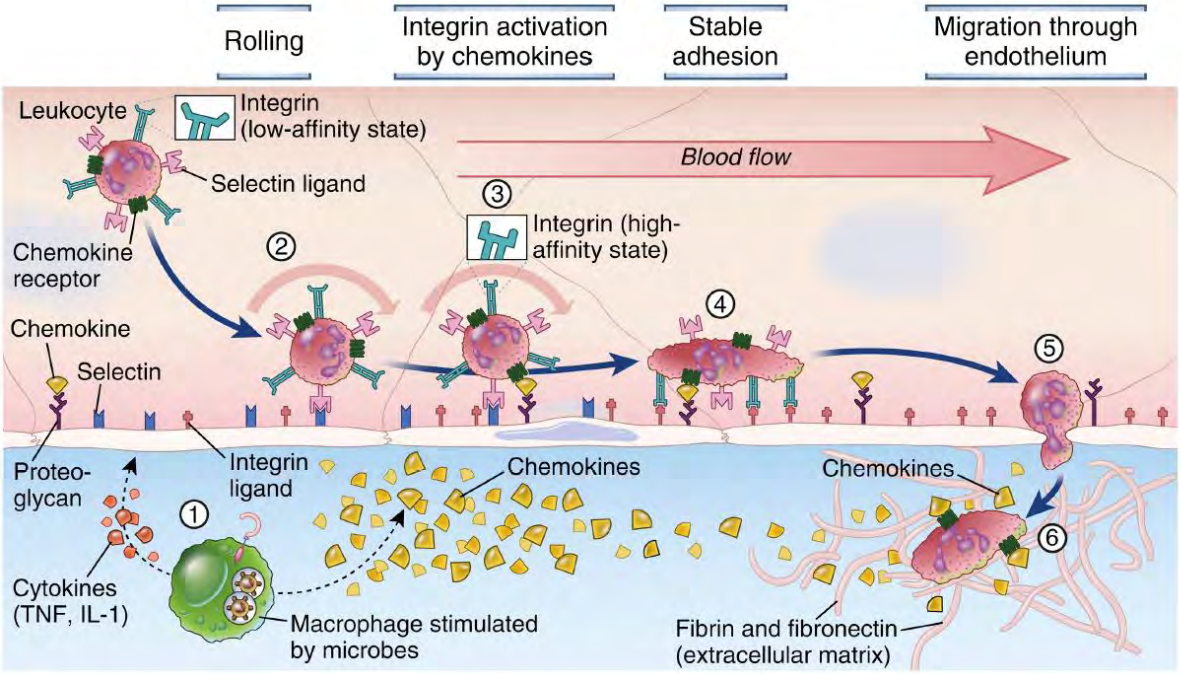
Dendritics



## Neutrophils (PMNs)

- the most abundant (50-70%) of circulating white blood cells
- Short life-span (18 hours to 4 days)
- arrive first at the site of inflammation
- migrate via chemotaxis (**C5a, IL-8**) toward site of inflammation
- **IL-17 (Th17)** promotes neutrophil action

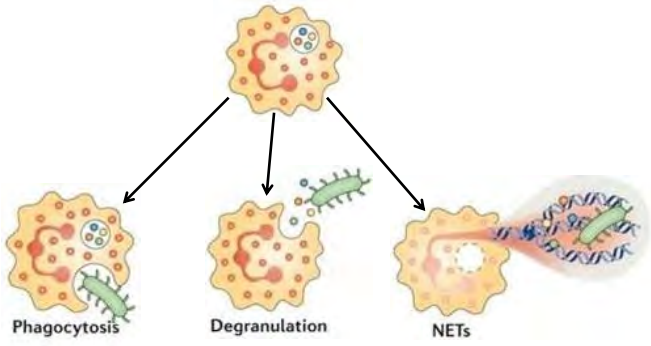



AK Abbas et al. Cellular and Molecular Immunology, 10th ed. 2021

## Granules of neutrophils

| Granule type | Protein |
|---|---|
| azurophilic granules (or "primary granules") | Myeloperoxidase (MPO)<br>Bactericidal/permeability-increasing protein<br>Defensins<br>Serine proteases neutrophil (elastase, cathepsin G) |
| specific granules (or "secondary granules") | Alkaline phosphatase<br>Lysozyme<br>NADPH oxidase<br>Collagenase<br>Lactoferrin<br>Cathelicidin (LL-37) |
| tertiary granules | Cathepsin , gelatinase |

## Neutrophils employ three strategies to contain and clear the infection

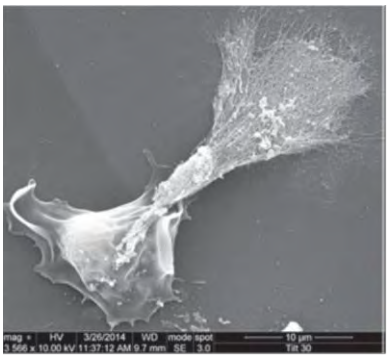



i. **Phagocytosis:** Involves the engulfment and subsequent elimination of microbes in specialized phagolysosome compartments.

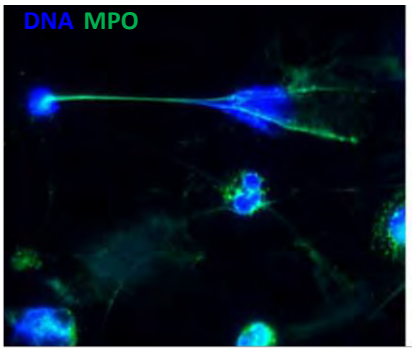
ii. **Degranulation:** Releases antimicrobial molecules in the vicinity of infection.

iii. **Neutrophil Extracellular Traps (NETs):** NETs are extracellular neutrophil-derived DNA fibers that trap and kill invading pathogens.

- NETs are composed **of citrullinated chromatin** and **granule constituents** (myeloperoxidase, elastase, defensins etc)
- NETs are released by neutrophils undergoing **phagocytosis** or after activation **by autophagy ROS and various inflammatory mediators**
- Implicated in several disorders including **infections/sepsis, thrombosis, SLE, RA, ANCA+ vasculitis, FMF, gout, psoriasis....**

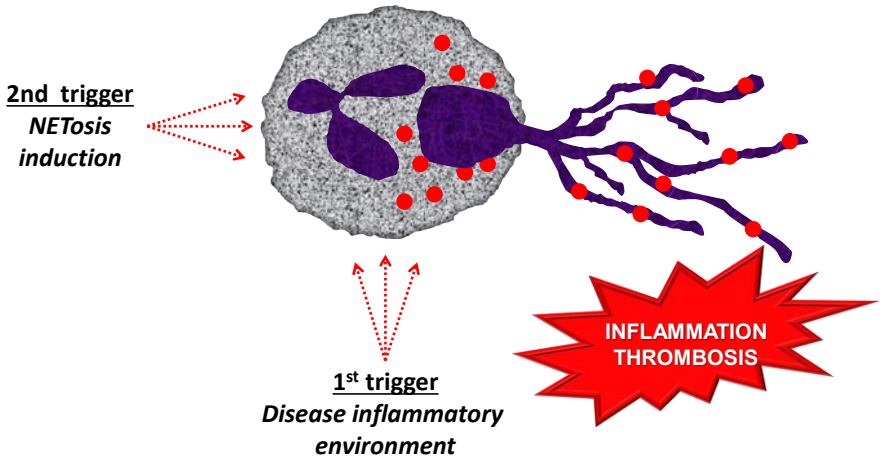


Barnado A et al. J. Leukoc. Biol. 2016




*Laboratory of Molecular Hematology, DUTH, Alexandroupolis, Greece*

## NET release (NETosis)
## "Two hits model"




Modified: Skendros et al. JACI 2017

## Excess NET formation can drive a variety of severe pathologies

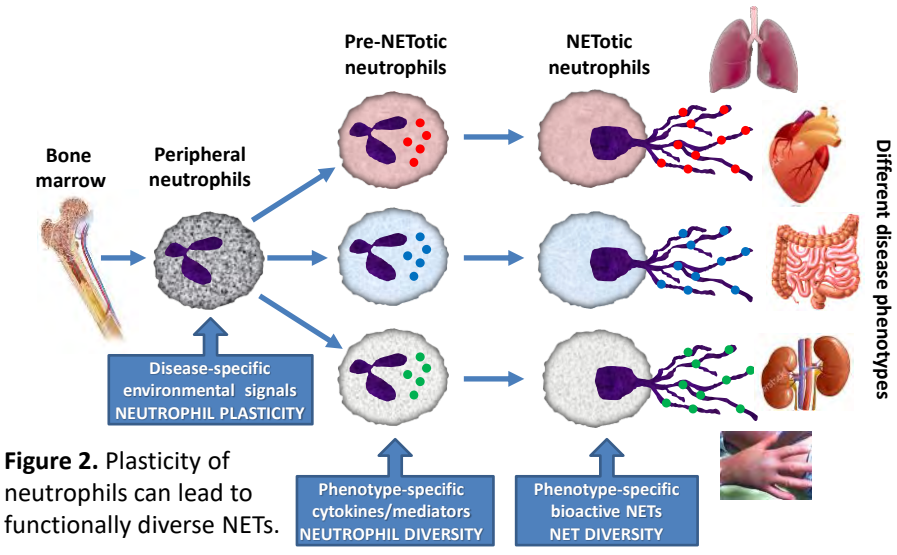
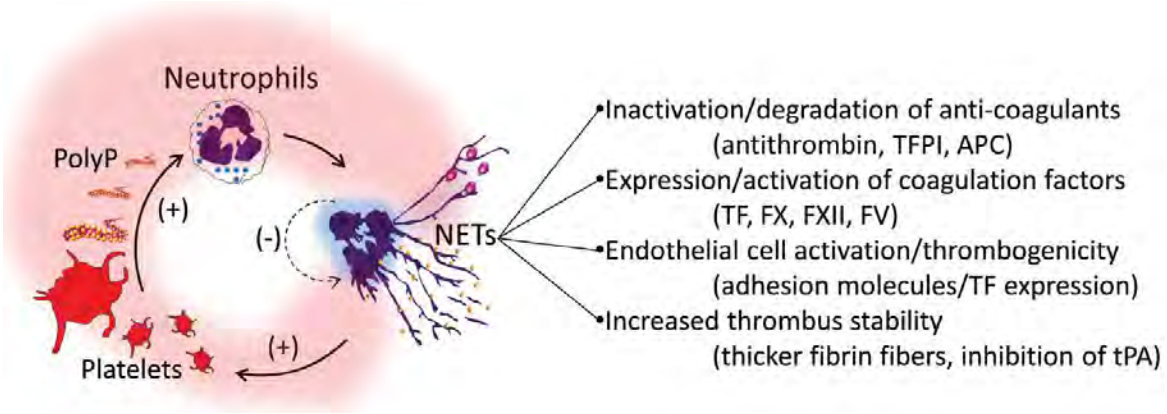



**Figure 2.** Plasticity of neutrophils can lead to functionally diverse NETs.

Skendros P et al. CYTONET Project

# Mechanisms of neutrophil extracellular trap (NET) thrombogenicity

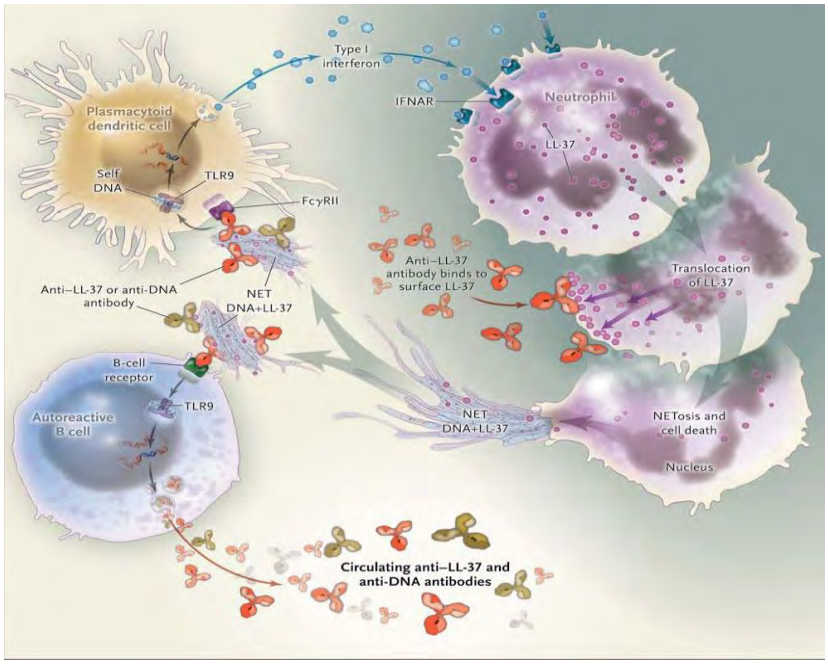



Stakos & Skendros. Thromb Haemost. 2020

# Proteins derived from NETs may serve as self-antigens and mediate organ damage in autoimmune diseases

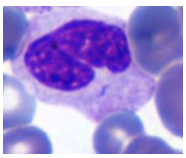
**Table 1 | NET proteins with potential roles in autoimmunity.**

| Protein | Present in disease-specific NETs (by IF) | Present in PMA-induced NETs (by proteomics*) | AutoAbs | Role(s) in auto-immunity |
|---|---|---|---|---|
| Histones | All (by definition) | Yes | SLE, Felty's | AutoAg in SLE and Felty's; pro-thrombotic |
| MPO | SLE, psoriasis, SVV, gout | Yes | SVV, SLE | AutoAg in SVV; oxidative stress? |
| Proteinase 3 | SVV | Yes | SVV, SLE | AutoAg in SVV |
| LL37 | SLE | No | SLE | Binds ICs to activate pDCs |
| HNP/α-defensins | SLE | Yes | SLE | Binds ICs; predisposes to CVD? |
| HMGB1 | SLE, gout | No | Unknown | Binds ICs; pro-inflammatory |
| IL-17 | SLE, psoriasis | No | SLE, psoriasis | Pro-inflammatory |
| C1q | SLE | No | SLE | Activates complement; protects from degradation? |
| Elastase | SLE, psoriasis | Yes | SLE | Unknown |
| Lactoferrin | Unknown | Yes | SLE | Unknown |
| Cathespin G | Unknown | Yes | SLE | Unknown |
| Calprotectin | Unknown | Yes | Unknown | Unknown |
| α-enolase | Unknown | Yes | SLE | Unknown |
| Catalase | Unknown | Yes | SLE | Oxidative stress? |

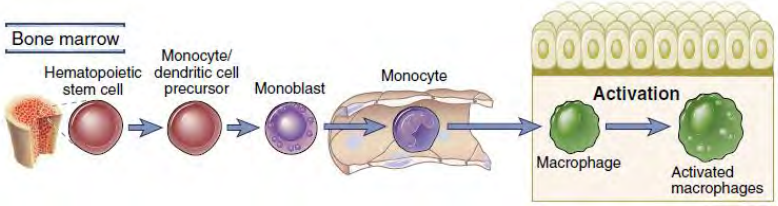



***NETs in Lupus, N Eng J Med* 2011**

## Monocytes-Macrophages (Mϕ)

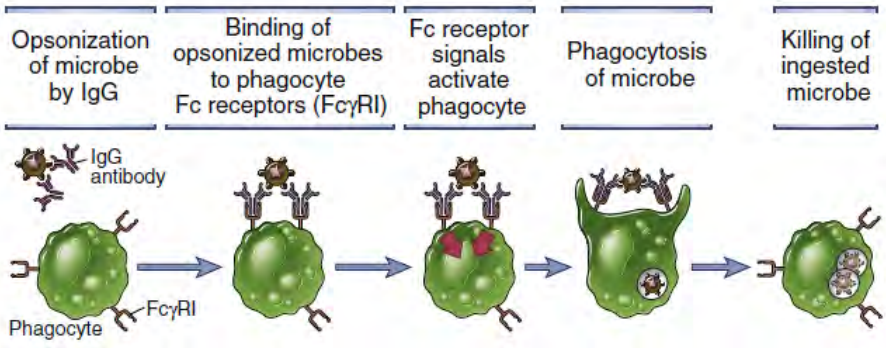


- **Monocytes** arise from the myeloid lineage in the bone marrow
- Circulate in the blood, and are recruited into tissues in inflammatory reactions, where they mature into **macrophages**
- **Phagocytosis**
- **Antigen presentation** **(APC: antigen-presenting cell)**

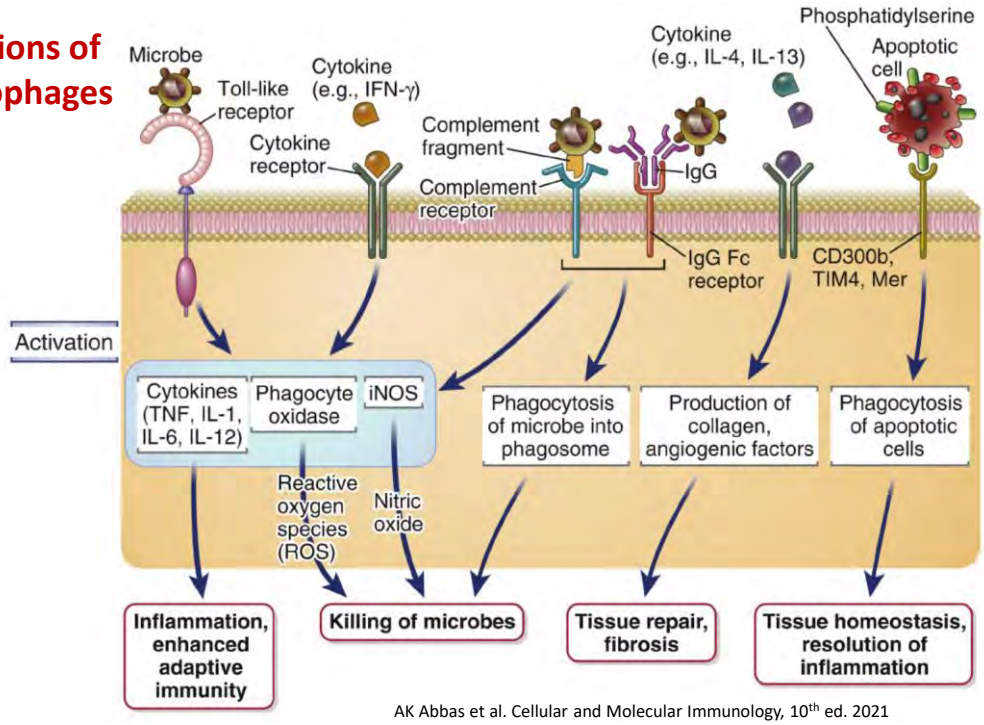


# Opsonization

- Robust phagocytosis requires opsonization
- The process of coating particles to promote phagocytosis
- Substances that perform this function (opsonins), including **antibodies (IgG)** and **complement (C3b)**
- **Fcγ** και **CR1** receptors, respectively

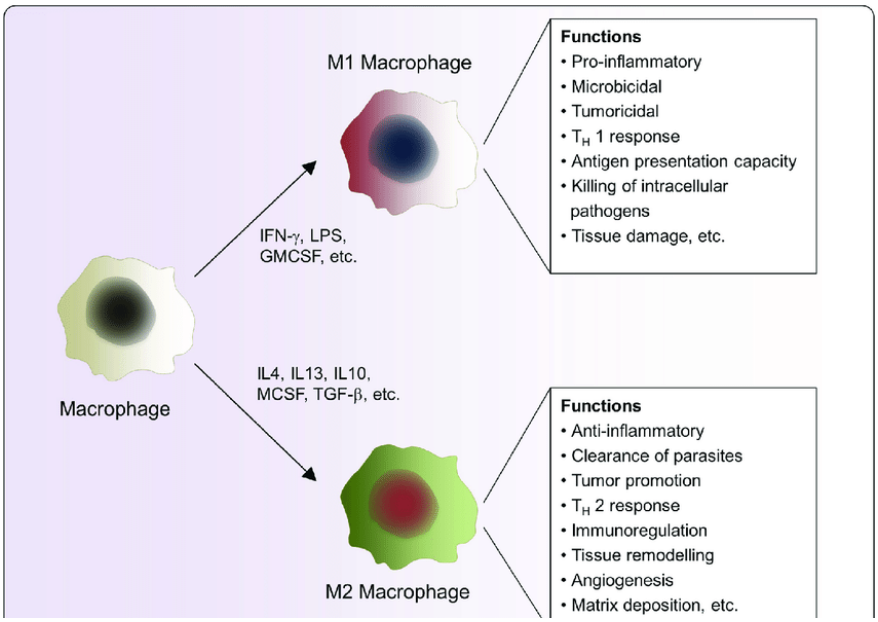



## Functions of macrophages



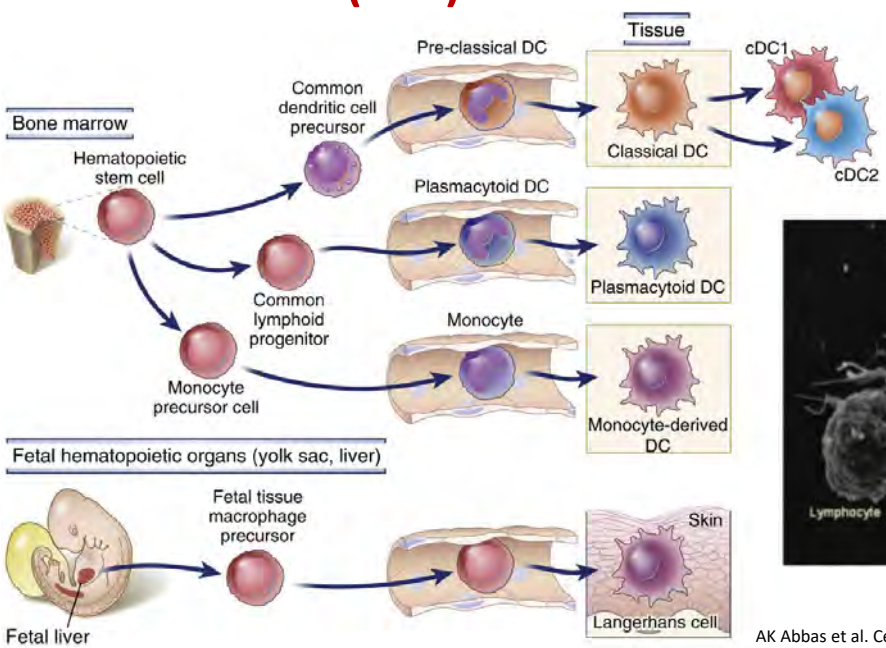

AK Abbas et al. Cellular and Molecular Immunology, 10th ed. 2021

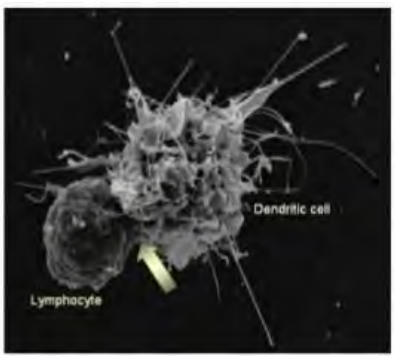
## Macrophage polarization and specific functions of M1 and M2 macrophages




Saqib U et al. Oncotarget 2018

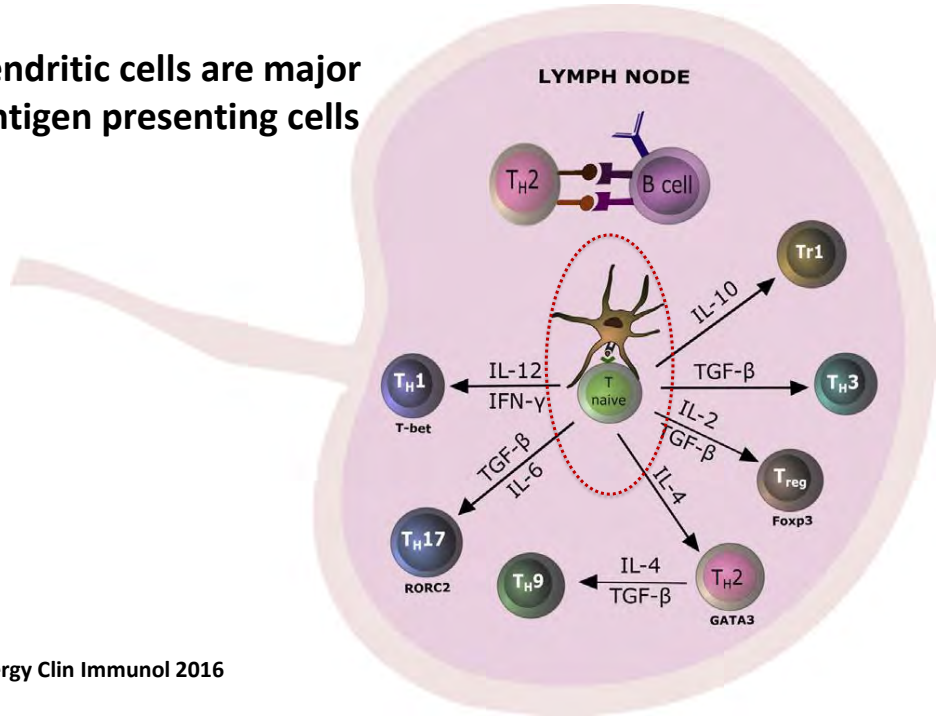
# Dendritic cells (DCs)



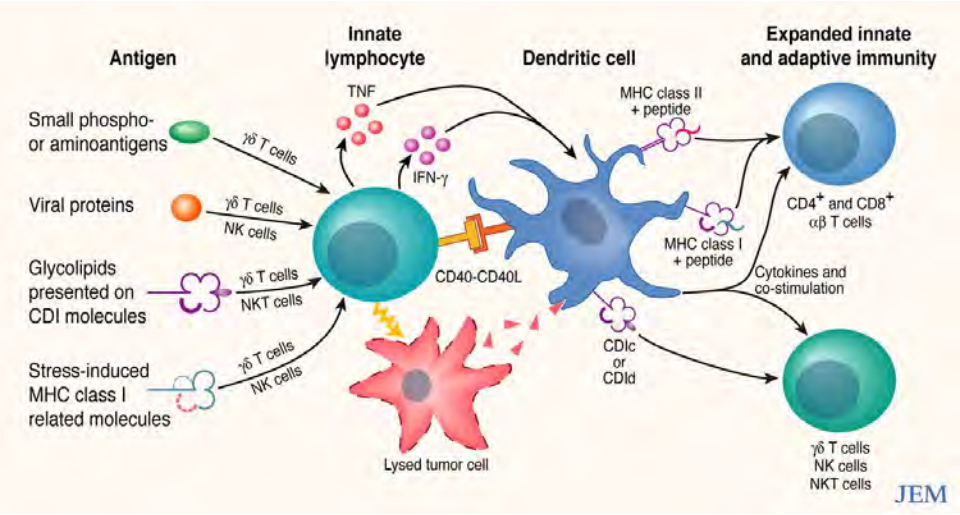

AK Abbas et al. Cellular and Molecular Immunology, 10th ed. 2021

## Dendritic cells are major antigen presenting cells




J Allergy Clin Immunol 2016

## Innate lymphocytes (Innate immunity lymphocytes, NK, NKT, γδT)

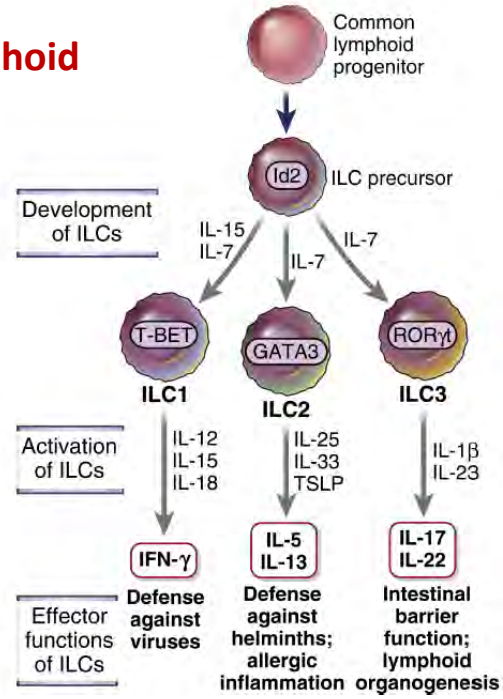



Münz C et al. J Exp Med 2005;202:203-207



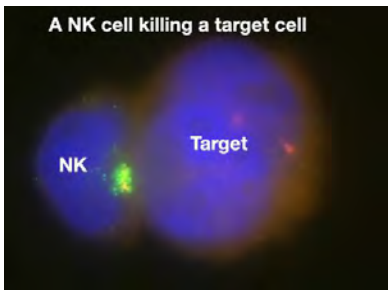
JEM

## Innate lymphoid cells (ILCs)




AK Abbas et al. Cellular and Molecular Immunology, 10th ed. 2021

## Natural Killers cells, NK (cytotoxic cells)

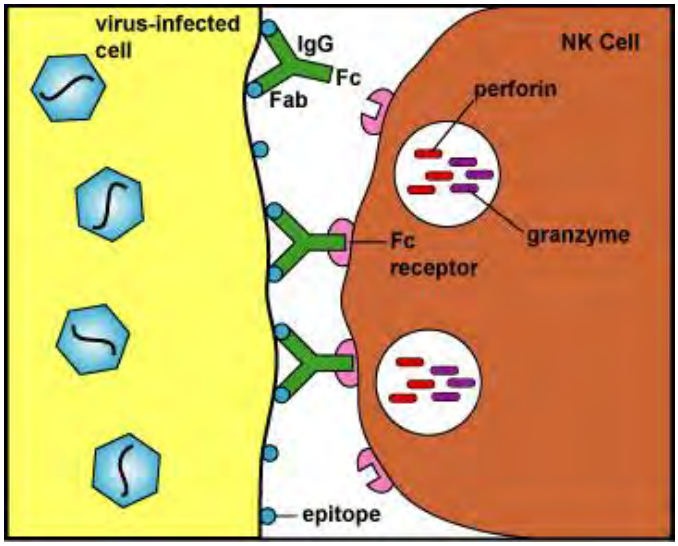



- **Antiviral immunity**

*recognize infected cells early in the course of infection, before adaptive immune responses have developed*

- **Anti- tumor immunity**

## **NK cells:** Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

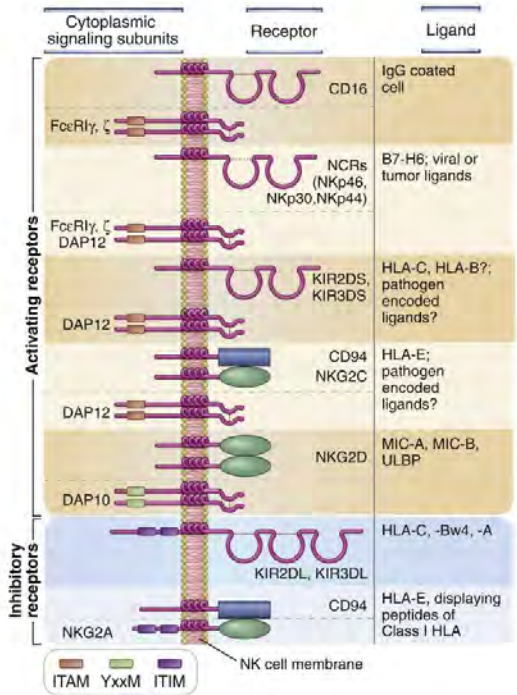



## NK receptors

activating

***NK cells distinguish infected and stressed cells from healthy cells, and cell function is regulated by a balanc between signals that are generated activating and inhibitory receptors***

inhibitory



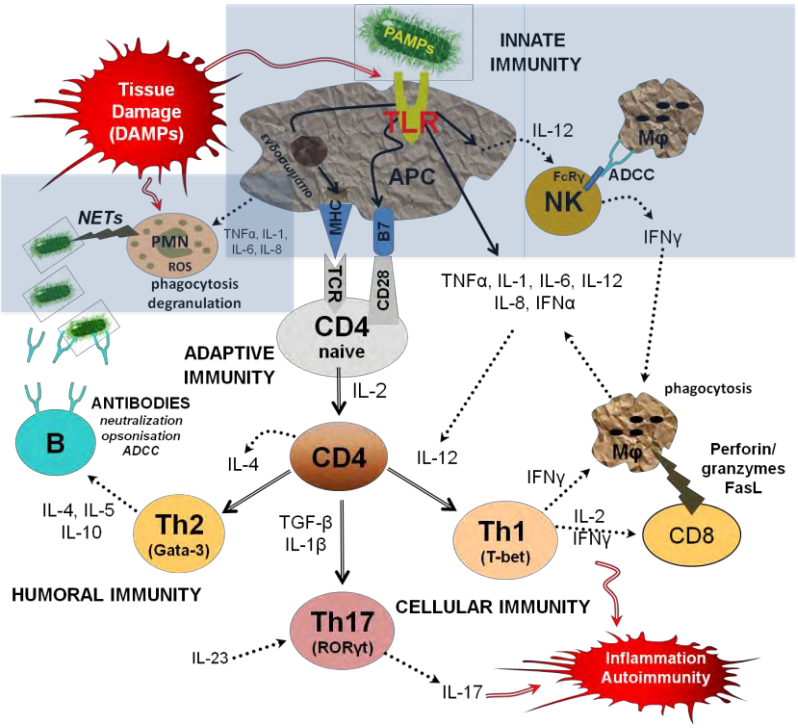
## Innate immune system……functions

- Recognizes molecular structures that are produced by **microbial pathogens** or **injured cells** by using:
  - PRRs (eg TLRs)
  - complement, other soluble mediators (eg CRP)

- Elimination of **microbes** and **damaged cells** and initiation the process of **tissue repair**

- Induction of **inflammation**, **activation of complement**, **coagulation**, **opsonisation/phagocytosis**

- Provides **alert signals to adaptive immunity** to stimulates adaptive immune responses, **influence the nature of the adaptive responses against different types of dangerous**
  - MHC-II, B7 (CD80)
  - IL-12



"…go forth, you young people, and tackle the really difficult problems in health…….you have all the tools, now we need individuals who have the will to work…"

**Charles Alderson Janeway, Jr. (1943 – 2003)**