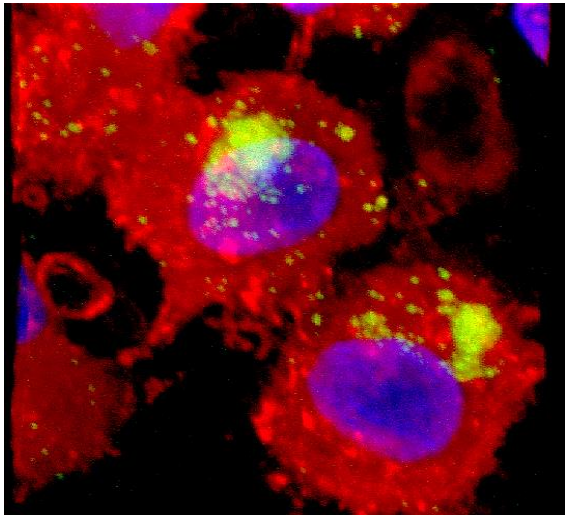
INNATE IMMUNITY

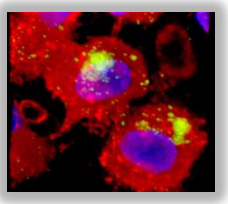


Priv.-Doz. Dr. Wilfried Posch

Univ.-Ass./Senior Scientist

Institute of Hygiene and Medical Microbiology

Medical University of Innsbruck



CONTENTS

Lecture: Innate Immunity

Humoral Innate Immunity
Cellular Innate Immunity
PRRs

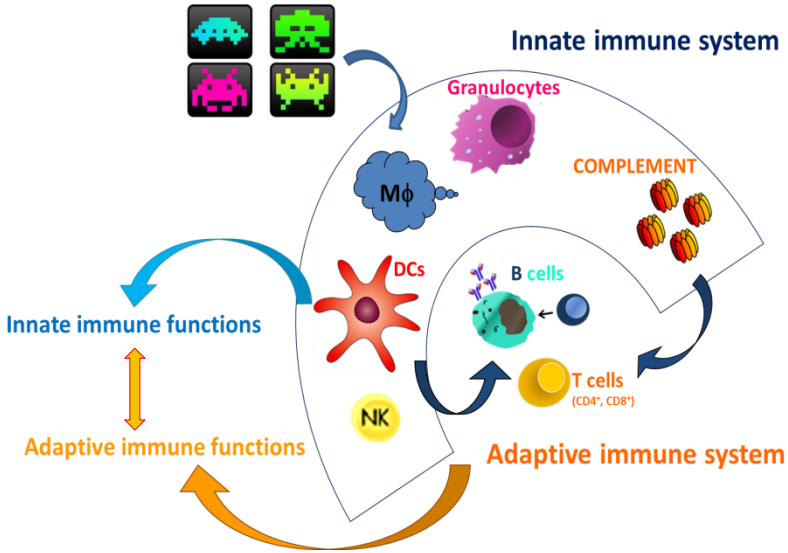
Journal Club:

'*Intracellular Complement Activation Sustains T Cell Homeostasis and Mediates Effector Differentiation*'
Liszewski MK et al., Immunity 2013

INNATE IMMUNITY

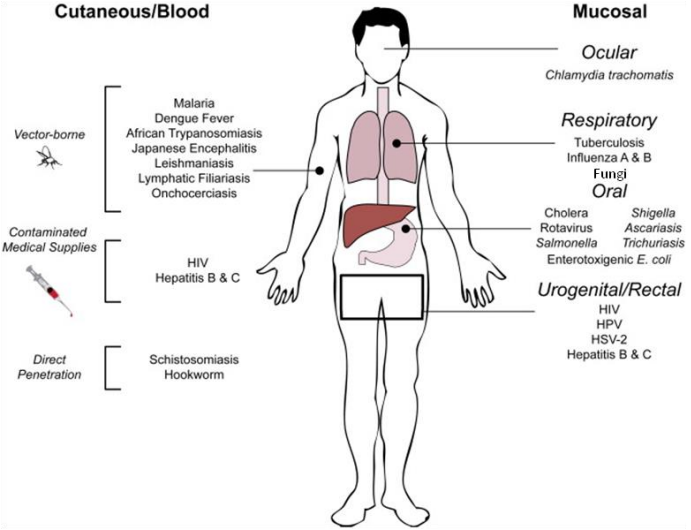
INNATE IMMUNITY: ALWAYS AT THE READY

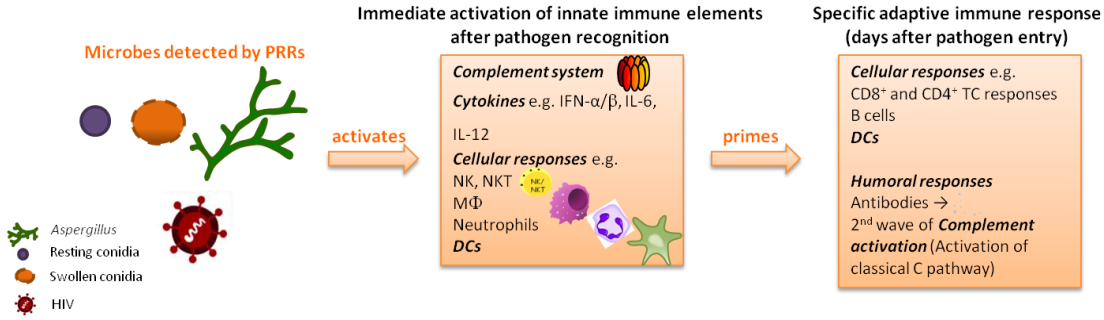
- when microbes break the surface borders (skin, mucosa, etc.) → innate immune responses are immediately activated

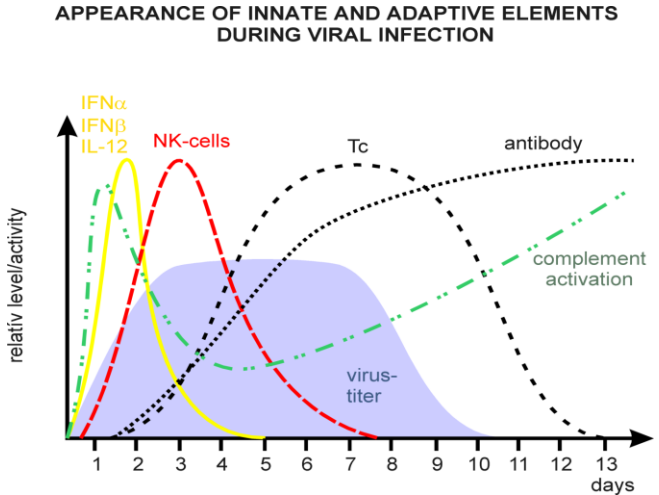


- humoral innate immune responses are spontaneously activated (***complement, cytokines, defensins***)
- co-ordinated and immediate recruitment of innate immune cells (***macrophages, neutrophils, NK cells, NKT cells, dendritic cells***) to the sites of infection and induction of defense mechanisms against the invader
- cells interact via pattern recognition receptors (***PRRs***) with microbes or their products and internalize these

INNATE IMMUNITY:
INFECTION ROUTES AND APPEARANCE OF IMMUNE ELEMENTS

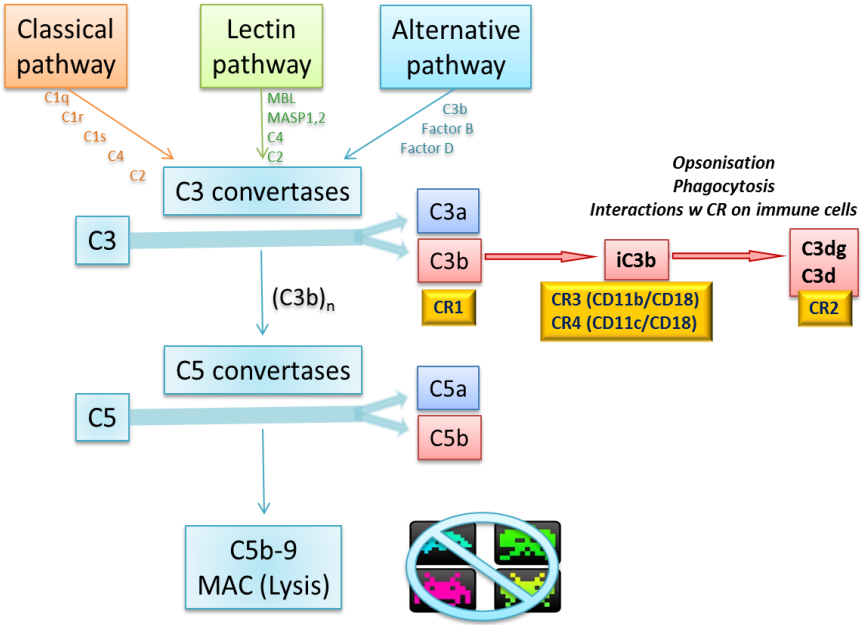






INNATE IMMUNITY: HUMORAL COMPONENTS

Complement system



Other soluble factors:

cytokines

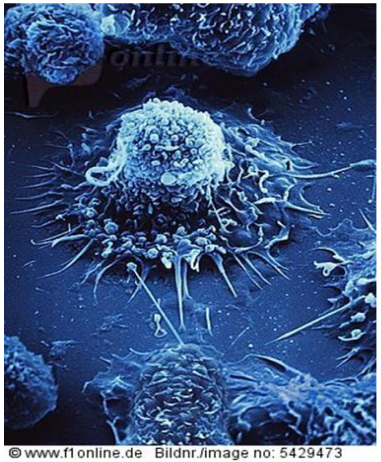
defensines

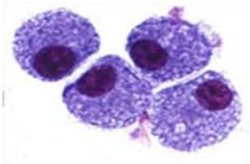
anaphylatoxins (C3a, C5a)

Impact on other immune responses/cells

INNATE IMMUNITY: CELLS, E.G.

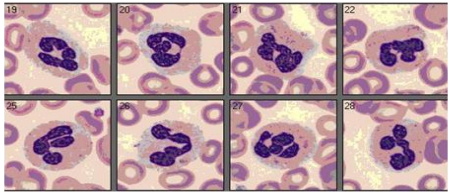
Makrophagen



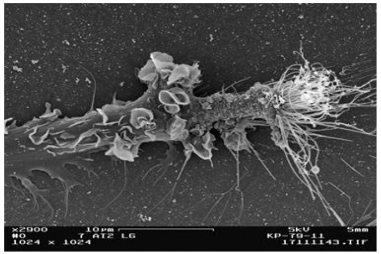


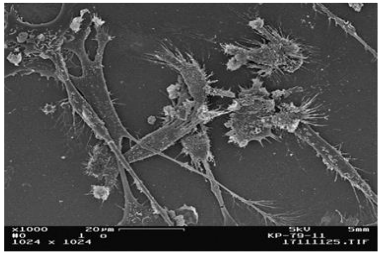
Neutrophile Granulozyten

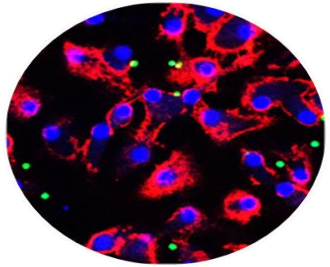
Dr. Volker Brinkmann,
Max-Planck Institut für Infektionsbiologie



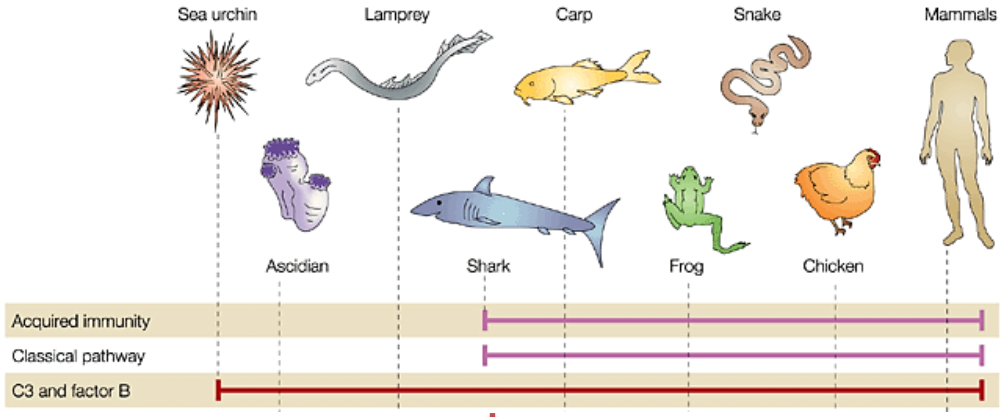
Dendritische Zellen







THE COMPLEMENT SYSTEM



Fujita T., Nat.Rev.Imm., 2002

Complement system

> Immediately activated upon entry of pathogen

Complement fragments coat the pathogen surface ***(Opsonisation)***

Destruction of the pathogen

Labelling of the pathogen

THE COMPLEMENT SYSTEM

- part of the immune system that helps or complements the ability of antibodies and phagocytic cells to clear pathogens from an organism

- comprised of more than 30 serum proteins and cell membrane receptors, mostly synthesized by the liver > *5%* of the globulin fraction of blood serum, act as *opsonins*

- circulate as inactive precursors and when stimulated proteases cleave specific proteins, which initiates an *amplifying cascade* of further cleavages > results in massive amplification of the response and activation of the cell-killing membrane attack complex (***MAC***)

- 3 biochemical ways of complement activation: ***Classical*** pathway, ***Lectin*** pathway, ***Alternative*** pathway

Classical Pathway

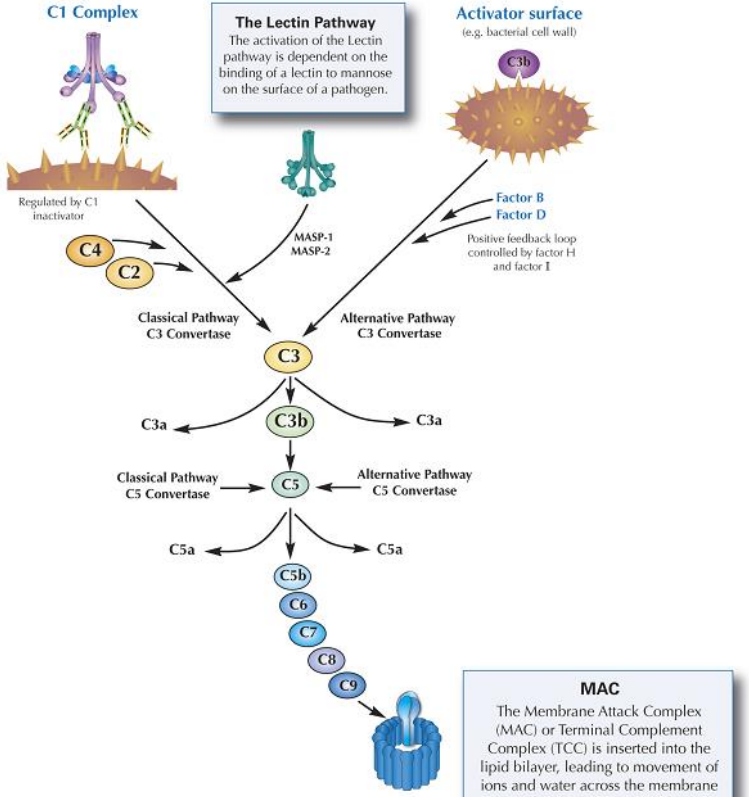
The Classical Pathway is activated by the Fc portion of an immunoglobulin in an antigen-antibody complex. It can also be activated by enzymes (e.g. trypsin and plasmin) and a variety of substances which include endotoxins, cell membranes and viruses.

Alternative Pathway

The Alternative Pathway does not depend on an antigen-antibody reaction in order to become active. Biological activators of this pathway include bacterial endotoxins, yeast cell walls, aggregated immunoglobulins and snake venom.

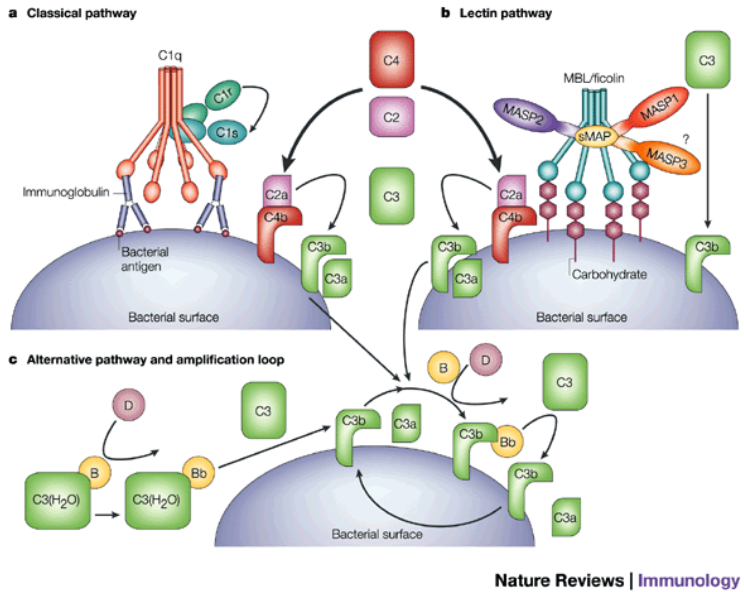
The Lectin Pathway

The activation of the Lectin pathway is dependent on the binding of a lectin to mannose on the surface of a pathogen.



MAC

The Membrane Attack Complex (MAC) or Terminal Complement Complex (TCC) is inserted into the lipid bilayer, leading to movement of ions and water across the membrane and ultimately to cell lysis.



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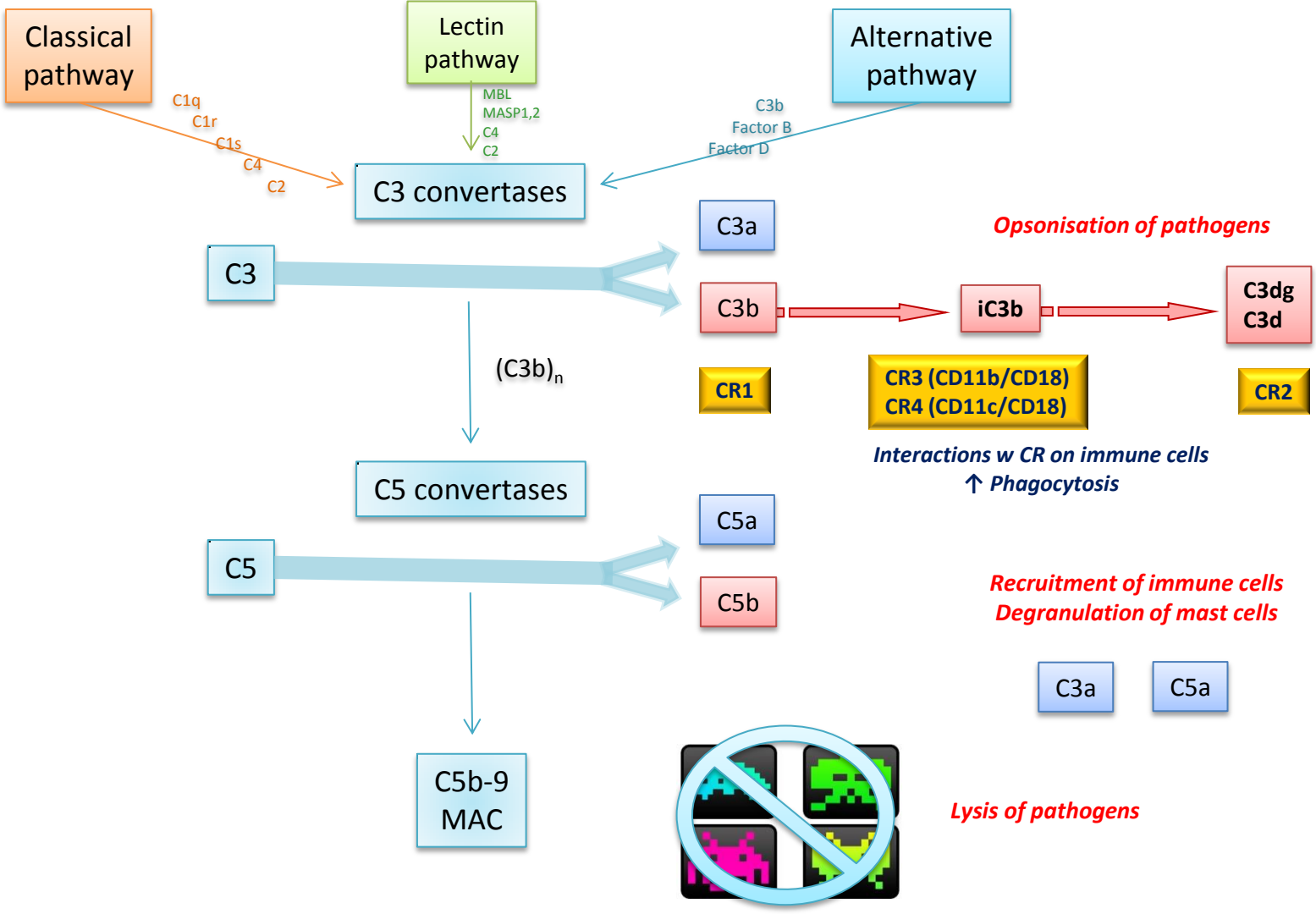
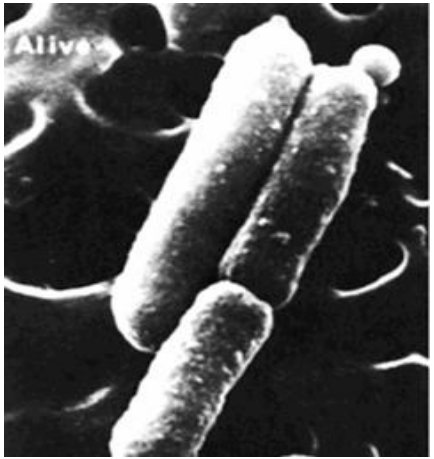
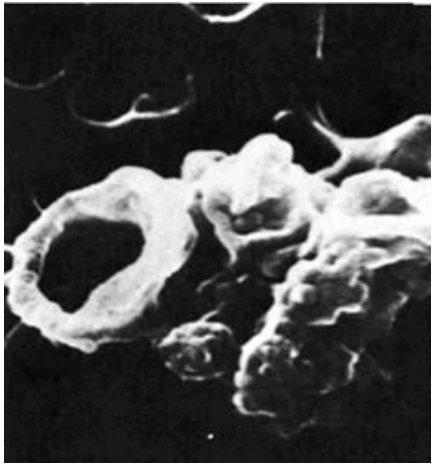

Classical pathway
C1q
C1r
C1s
C4
C2
Lectin pathway
MBL
MASP1,2
C4
C2
Alternative pathway
C3b
Factor B
Factor D
C3 convertases
C3
C3a
C3b
iC3b
C3dg
C3d
Opsonisation of pathogens
CR1
CR3 (CD11b/CD18)
CR4 (CD11c/CD18)
CR2
Interactions w CR on immune cells
↑ Phagocytosis
(C3b)n
C5 convertases
C5
C5a
C5b
Recruitment of immune cells
Degranulation of mast cells
C3a
C5a
C5b-9
MAC
Lysis of pathogens

COMPLEMENT-MEDIATED LYSIS OF BACTERIA



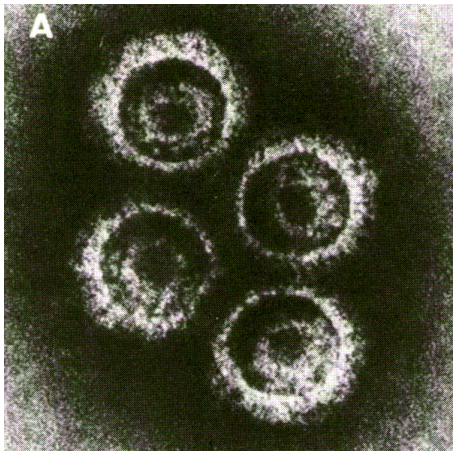
Intact E.coli



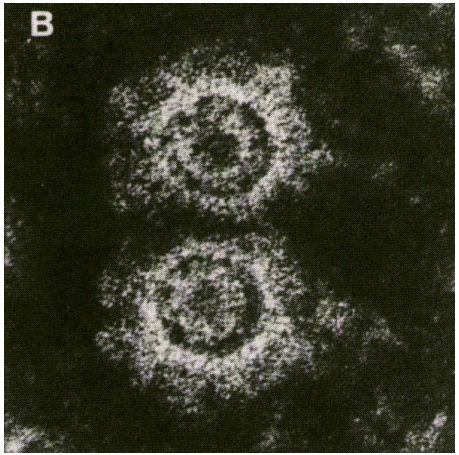
E.coli killed by action of complement

Schreiber et al, J.Exp.Med.149, 870
Scanning electron micrographs before and after killing by complement.

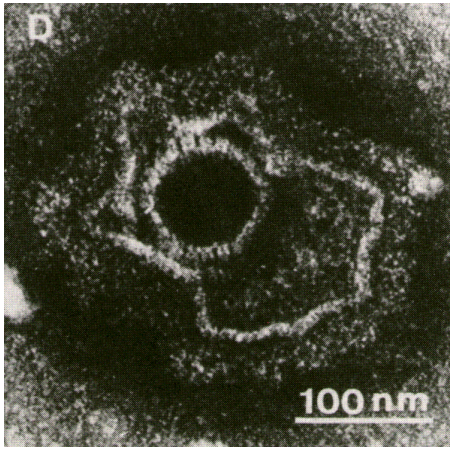
COMPLEMENT-MEDIATED LYSIS OF VIRUSES



EBV was incubated in buffer (A),

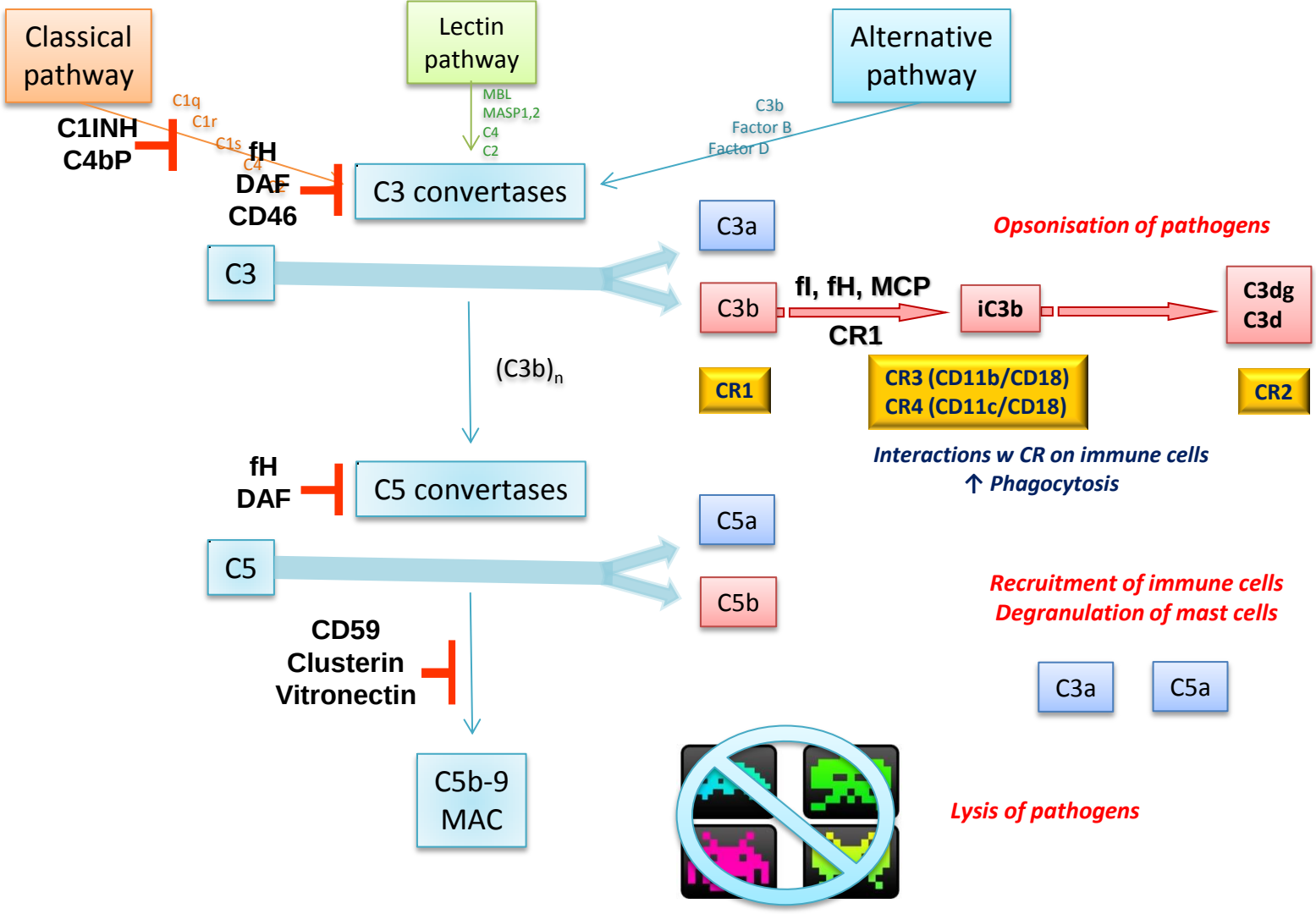


Antibodies (B)



Complement and antibodies (C)

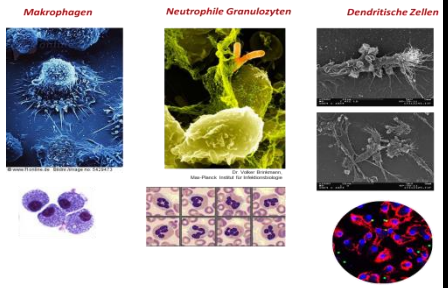
Nemerow and Cooper, 1981, J. Immunol. 127;273
EM negative staining; final magnification 1:125,000


Classical pathway
Lectin pathway
Alternative pathway
C1q
C1r
C1s
C4
C2
MBL
MASP1,2
C4
C2
C3b
Factor B
Factor D
C1INH
C4bP
fH
DAF
CD46
C3 convertases
C3a
C3
C3b
fI, fH, MCP
CR1
iC3b
C3dg
C3d
CR1
CR3 (CD11b/CD18)
CR4 (CD11c/CD18)
CR2
(C3b)n
fH
DAF
C5 convertases
C5a
C5
C5b
CD59
Clusterin
Vitronectin
C5b-9
MAC
Opsonisation of pathogens
Interactions w CR on immune cells
↑ Phagocytosis
Recruitment of immune cells
Degranulation of mast cells
C3a
C5a
Lysis of pathogens

DENDRITIC CELLS AND OTHER INNATE IMMUNE CELLS EXPRESS PRR$_S$ RECOGNIZING PATHOGENS

- PRRs: encoded in genome, not subject to rearrangement or variation
- PRRs: molecular sensors of infection on critical immune cells, i.e. DCs and macrophages
- but also other cells, i.e. epithelial cells, coming in contact with pathogens express subsets of PRRs

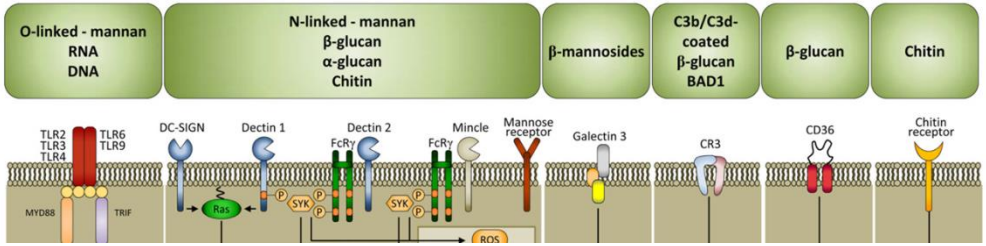
How can PRRs recognize pathogens from diverse families with different biology and patterns of infection in the absence of functional re-arrangement?



PRRs

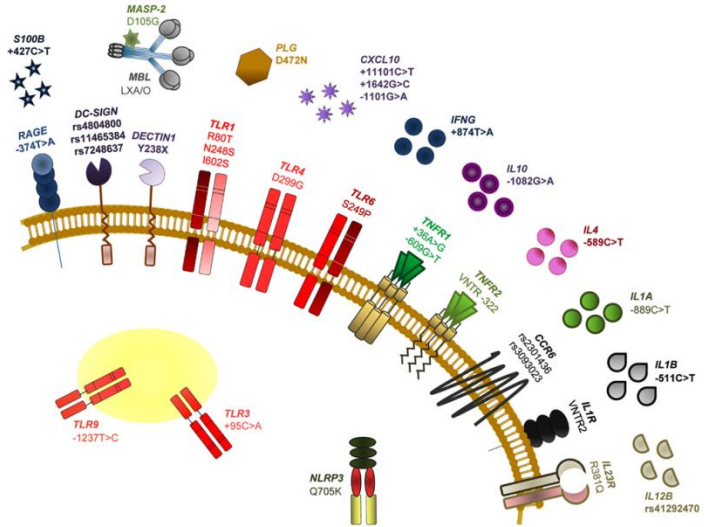
How can PRRs recognize pathogens from diverse families with diverse biology and patterns of infection in the absence of functional re-arrangement?

1)



Innate IS recognizes **PAMP**s (pathogen-associated molecular patterns) > components common to many pathogens

2)



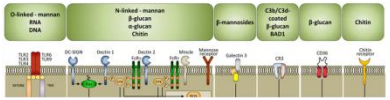
Secondly > **number** of **receptor families**, and many receptors in each family in extra- and intracellular spaces >

By having multiple sites for detection of diverse targets, it is unlikely that any given pathogen will be able to evade all of the levels of detection.

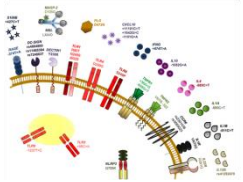
PRRs

How can PRRs recognize pathogens from diverse families with diverse biology and patterns of infection in the absence of functional re-arrangement?

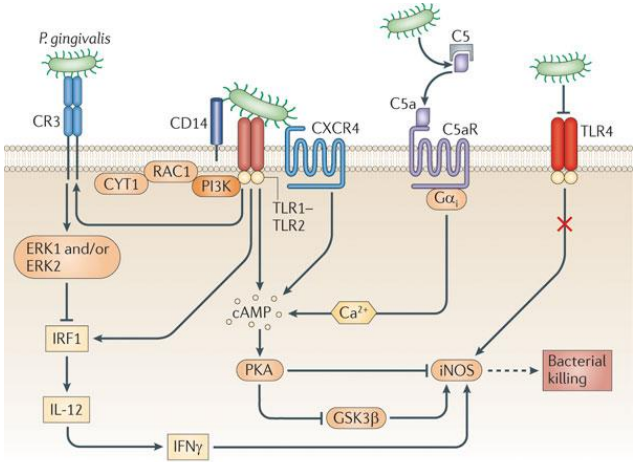
1)



2)



3)



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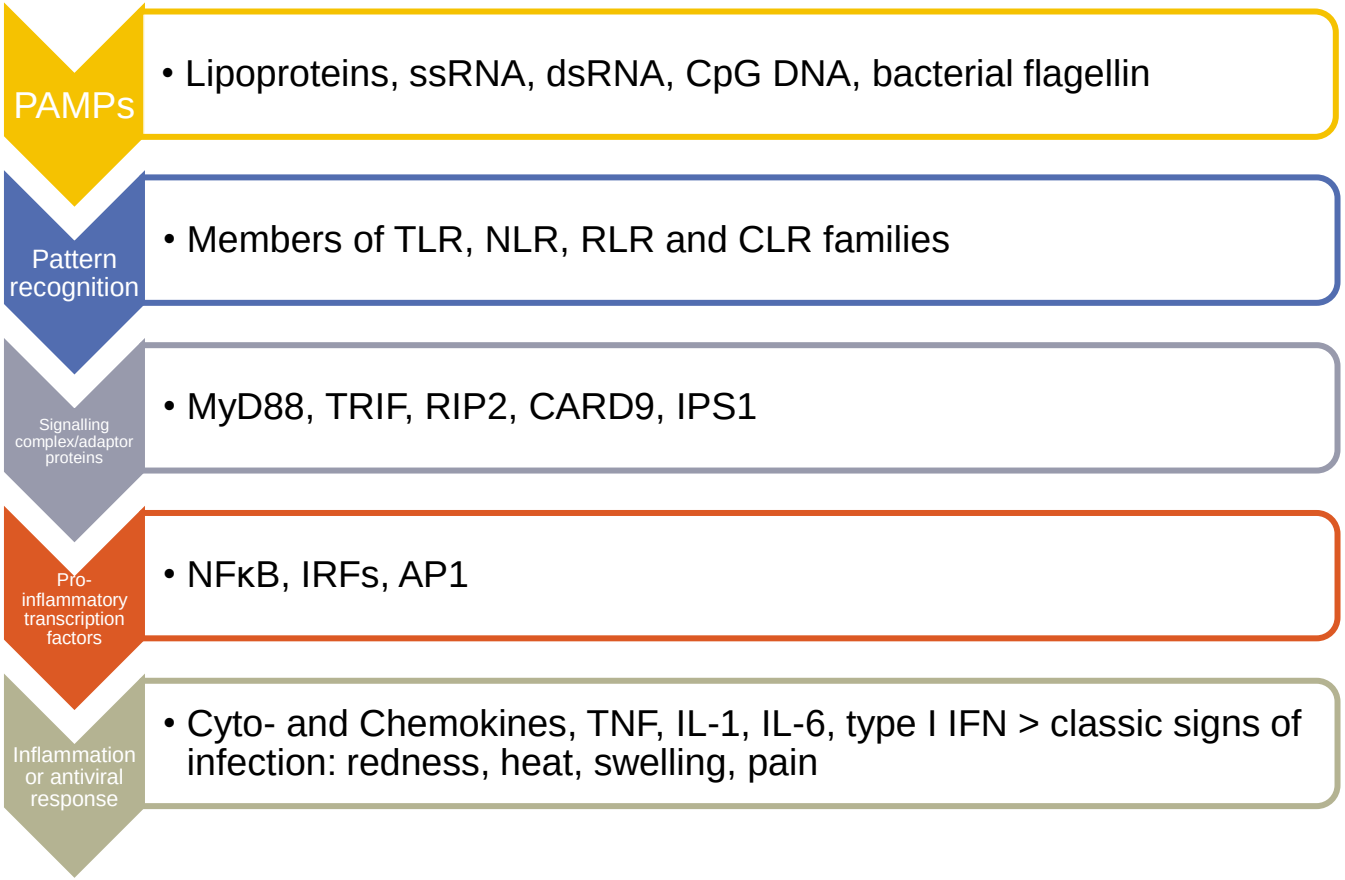
Extensive ***receptor cross-talk*** and ***communication*** between the ***signaling*** pathways > **co-ordinated** response to pathogen infection

PRRs

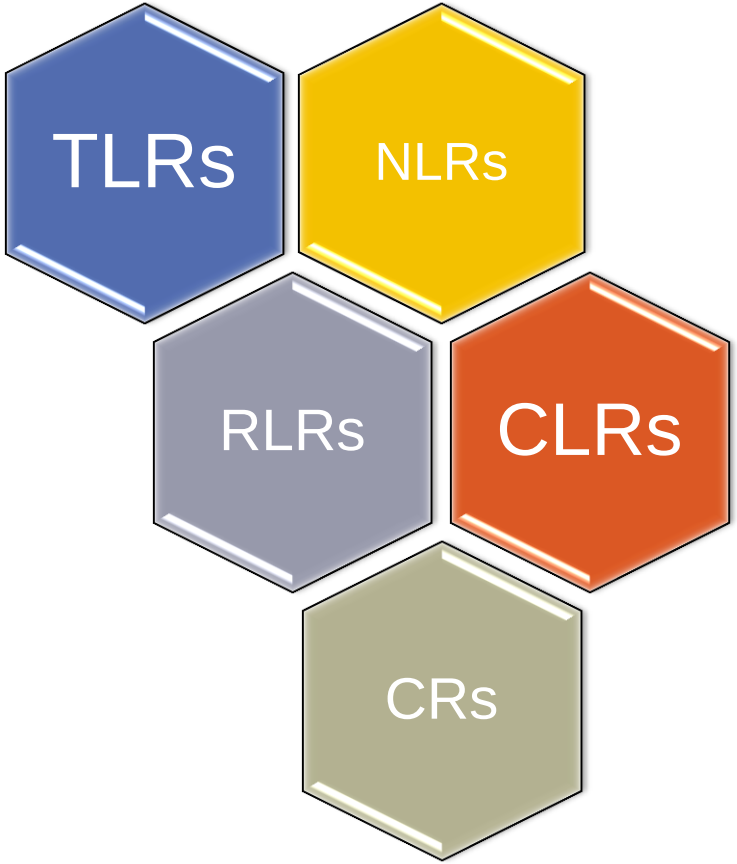
- Innate IR mediated via humoral components or PRRs: designed to induce ***inflammation*** at the site of infection, ***recruit*** inflammatory cells and mediators and begin to potentiate the adaptive immune system.

Stimulatory Pathogen Associated Molecular Pattern (PAMP)	Pattern Recognition Receptor (PRR)	Signalling Adapter Protein	Transcriptional or Cellular Pathway Activated
Toll-like receptors (TLRs)			
Bacterial cell wall components	TLR2 homo/heterodimers	MyD88	NFκB / AP1
LPS	TLR4 (plasma membrane)	MyD88	NFκB / AP1
LPS	TLR4 (endosome)	TRIF	IRF3 / NFκB / AP1
Flagellin	TLR5	MyD88	NFκB / AP1
dsRNA	TLR3	TRIF	IRF3 / NFκB / AP1
ssRNA	TLR7	MyD88	IRF7 / NFκB
Nod-like receptors (NLRs)			
iE-DAP	NOD1	RIP2	NFκB
MDP	NOD2	RIP2/CARD9	NFκB / AP1
e.g. Pore-forming toxins, nucleic acid	NLRP3	ASC	Caspase-1 activation
Retinoic acid-inducible gene I-like receptors (RLRs)			
dsRNA	RIG-I	MAVS	IRF3 / AP1 / NFκB
C-type lectin receptors (CLRs)			
β-glucans	Dectin-1	Syk	NFκB

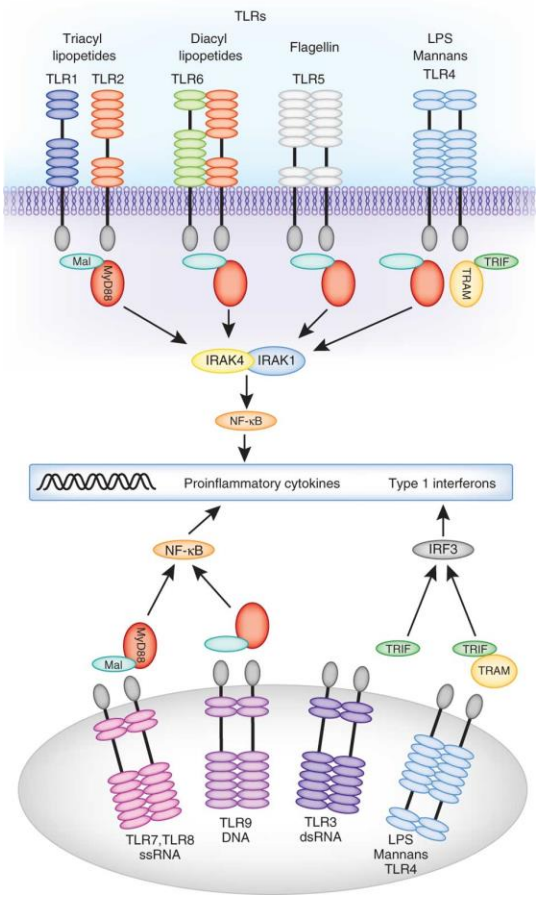
PRR$_S$ > OVERVIEW OF INNATE SIGNALING AND COMPONENTS



PRR FAMILIES



TLRs



- class of proteins that play a key role in the innate immune system
- single, membrane-spanning receptors usually expressed in sentinel cells
- recognize structurally conserved molecules derived from microbes
- breach of physical barriers by microbes (e.g. skin or intestinal tract mucosa) > recognition by TLRs
- TLRs recognize structurally conserved molecules derived from microbes

http://www.nature.com/ni/journal/v13/n6/fig_tab/ni.2284_F1.html

TLRs

- name from their similarity to the protein coded by the **toll** gene identified in *Drosophila*
  > researchers were so surprised that they spontaneously shouted out in German
  "Das ist ja toll!"

- TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13

Recognition of different antigens by TLRs:

TLR-1*:- *Bacterial lipoprotein and peptidoglycans

TLR-2:- Bacterial peptidoglycans

TLR-3*:- *Double stranded RNA

TLR-4:- Lipopolysaccharides

TLR-5*:- *Bacterial flagella

TLR-6:- Bacterial lipoprotein

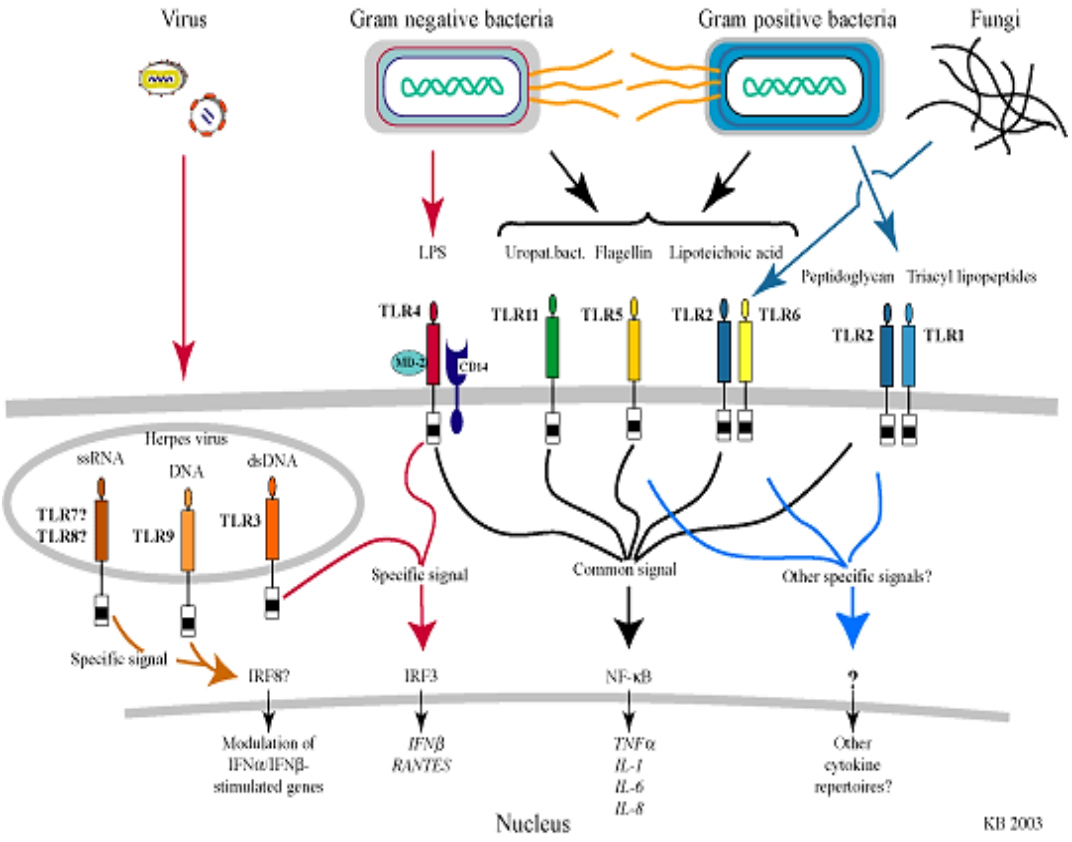
TLR-7*:- *Single stranded RNA

TLR-8:- Single stranded RNA

TLR-9*:- *CpG DNA

TLR-10:- Unknown

TLRs - Recognition



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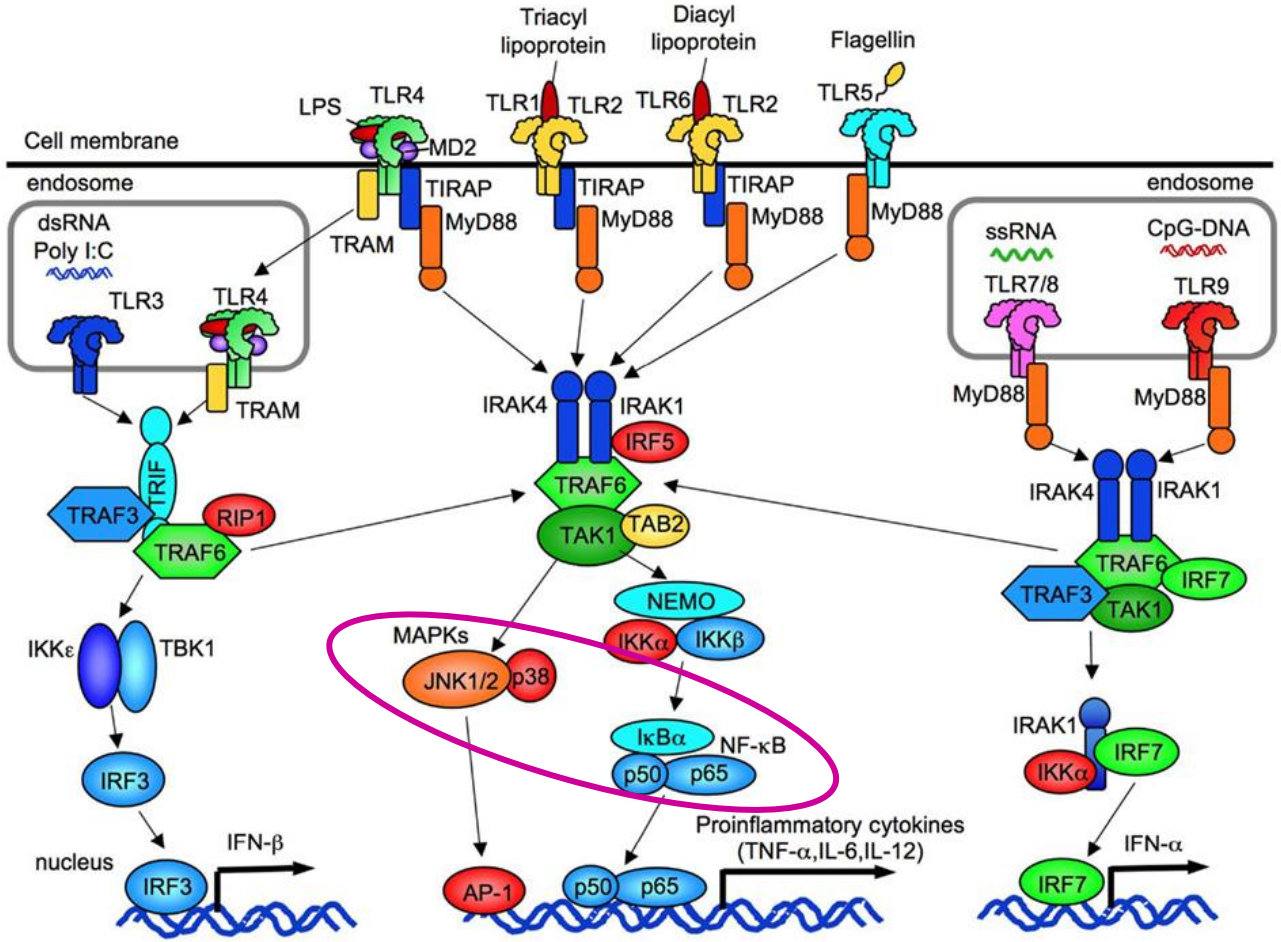
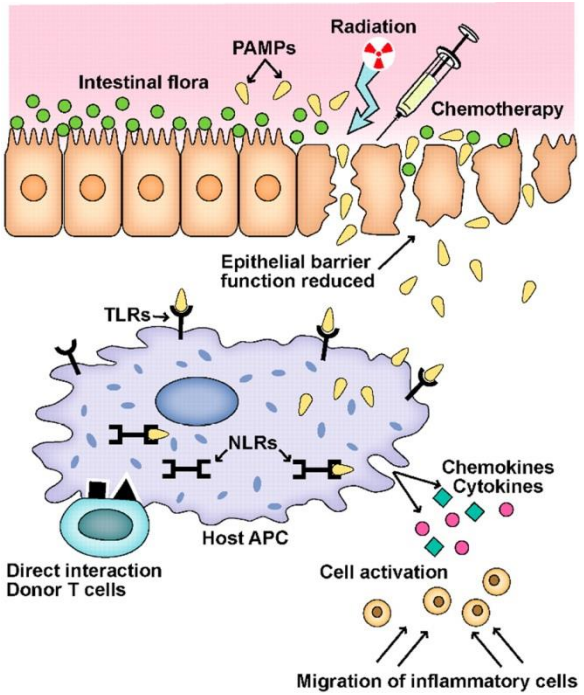
TLR-10:- Unknown

TLR - Signaling



NLRs

- nucleotide-binding oligomerization domain receptors or NOD-like receptors
- recognize intracellular PAMPs
- can co-operate with TLRs
- regulate inflammatory and apoptotic response
- expressed in lymphocytes, MΦ, DCs, and non-immune cells, e.g. epithelium



NLRs

3 domains:

NBD (nucleotide binding domain), LRR (leucine-rich repeat), variable N-terminal interaction domain

ATP-dependent oligomerization

Sensing presence of ligand

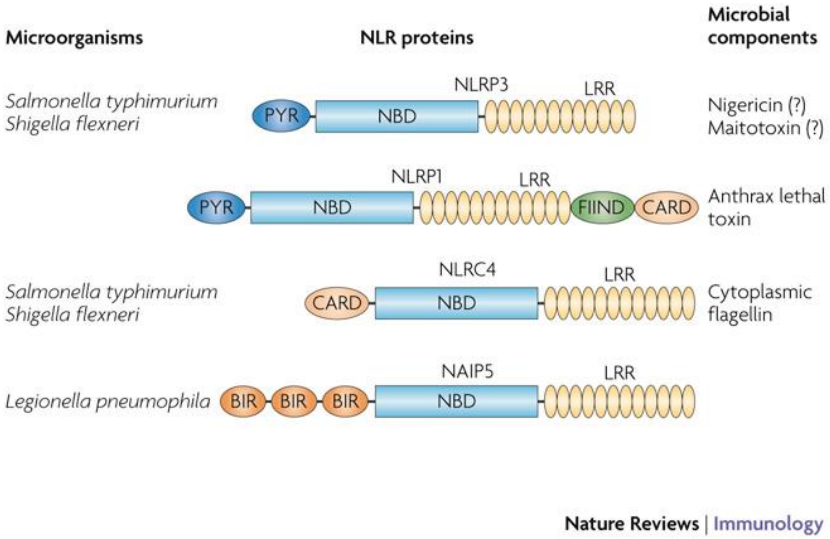
Responsible for homotypic protein-protein interaction

Can consist of:

CARD (caspase-recruitment domain)

PYR (pyrin domain)

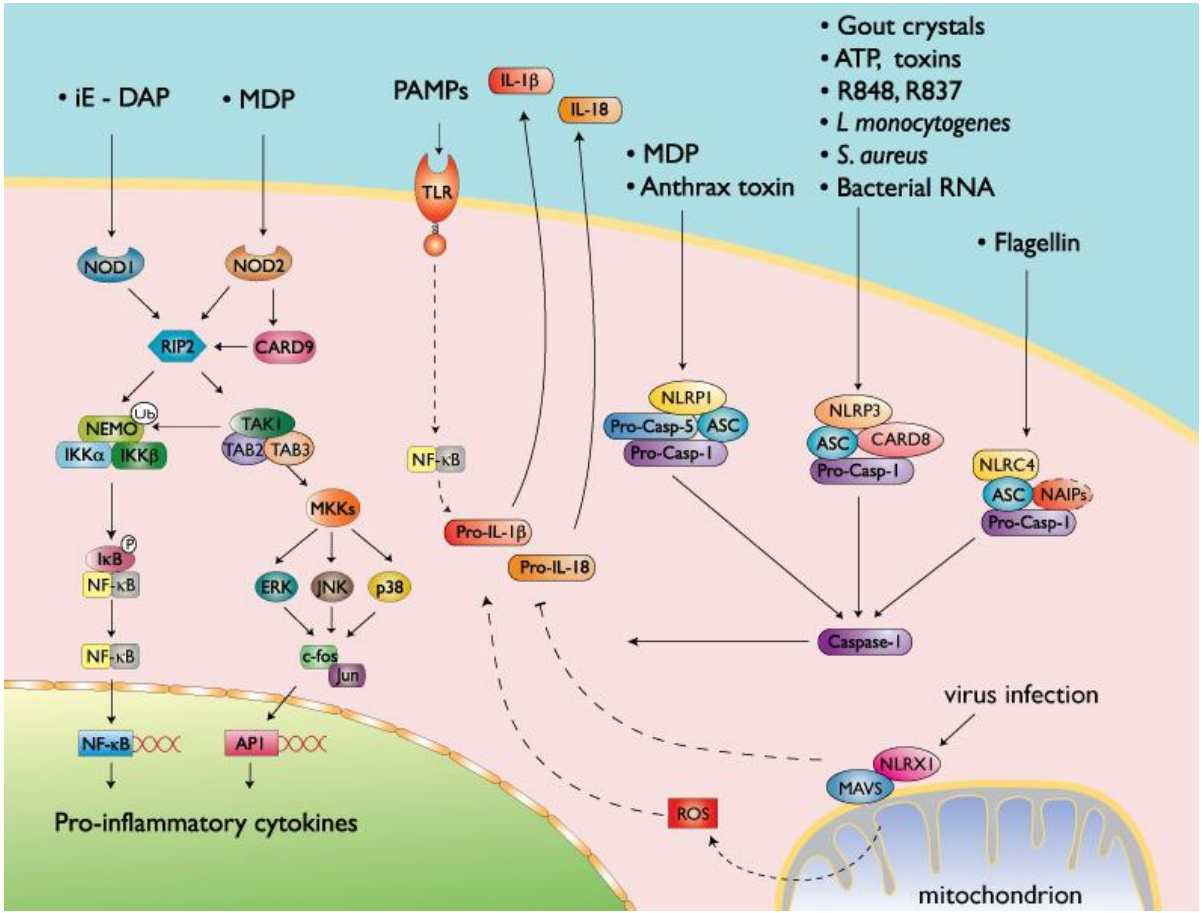
BIR (baculovirus inhibitor of apoptosis repeat)



http://www.nature.com/nri/journal/v8/n5/fig_tab/nri2296_F3.html

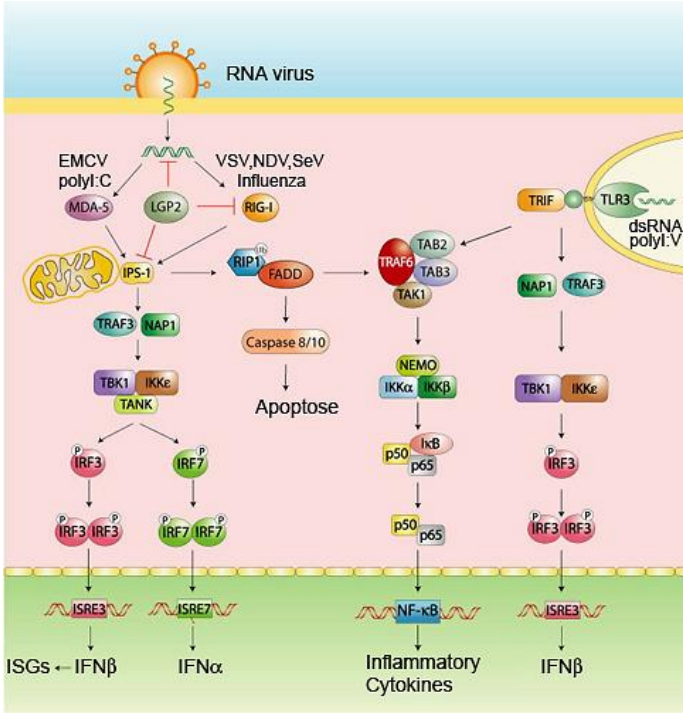
A caspase-1-dependent form of cell death called **pyroptosis** is thought to be mediated by NLRP1 in response to anthrax lethal toxin

NLR Signaling



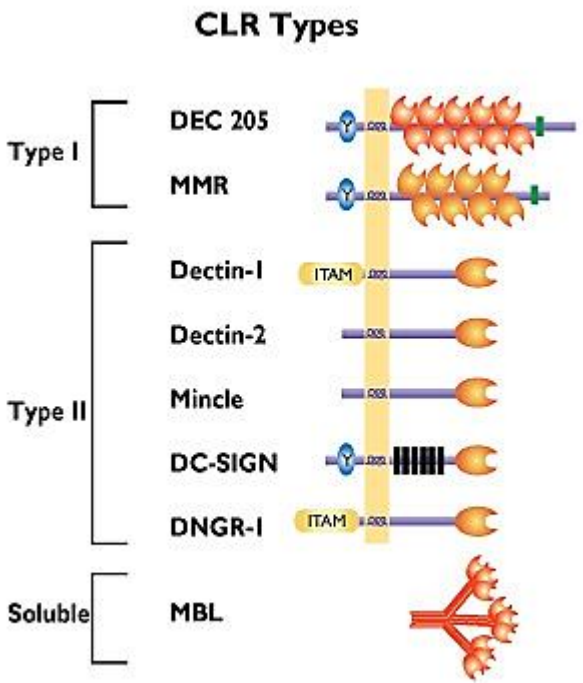
RLRs - retinoic acid-inducible gene I-like receptors

- small family of PRRs > **intracellular RNA**
- **sensors** of viral replication through direct interaction with **dsRNA**, which is produced by RNA viruses to form their genome (dsRNA viruses) or as a part of their replication cycle
- **RIG-I:** short uncapped dsRNA or ssRNA, **MDA5:** dsRNA
- RIG-I and MDA5 induce cellular response via **CARDs** (caspase recruitment domains), resulting in expression of **type I IFNs** and **pro-inflammatory cytokines** (IL-1β, IL-18)
- robust antiviral response via RLRs



http://www.invivogen.com/review-viral-dsrna-signaling

CLRs – C-type lectin receptors

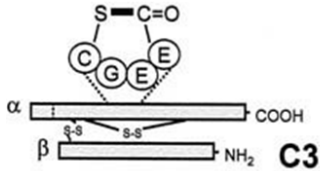


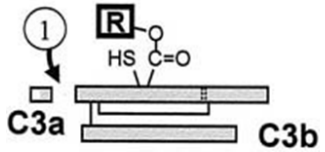
- comprise large family of PRRs
- bind to **carbohydrates** in a calcium-dependent manner
- based on molecular structures > **3 types of CLRs**: **Type I** (DEC-205, MR), **Type II** (dectins, mincle, DC-SIGN), **soluble** (MBL)
- involved in **fungal recognition** and **modulation** of the innate immune response
- expressed by most cell types including **macrophages** and dendritic cells (**DCs**) > internalize glycoproteins and microbes to clear and present Ag to T lymphocytes

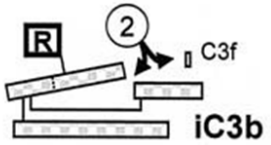
http://www.invivogen.com/review-clr

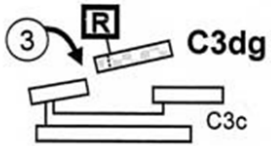
COMPLEMENT RECEPTORS – CR_S

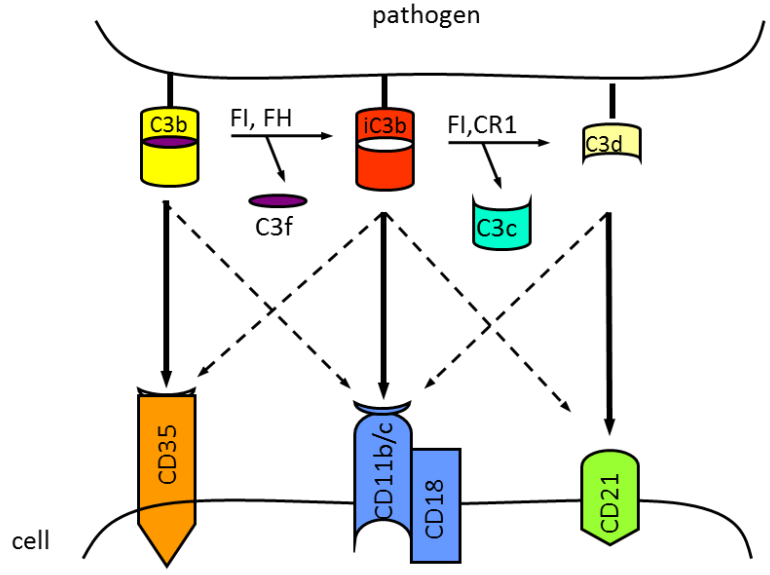
CR1 (CD35), 2 (CD21), 3 (CD11B/CD18), 4 (CD11C/CD18)











	CR1	CR3	CR2
Granulocytes	+	+	-
Monocytes	+	+	-
Macrophages	+	+	-
Dendritic cells	+	+ plus CR4 (CD11c/CD18)	-
Erythrocytes	+	-	-
T cells	+	+/-	act. +
B cells	+	-	+
FDC	+	+	+

Intracellular Complement Activation Sustains T Cell Homeostasis and Mediates Effector Differentiation

M. Kathryn Liszewski, Martin Kolev, Gaelle Le Friec, Marilyn Leung, Paula G. Bertram, Antonella F. Fara, Marta Subias, Matthew C. Pickering, Christian Drouet, Seppo Meri, T. Petteri Arstila, Pirkka T. Pekkarinen, Margaret Ma, Andrew Cope, Thomas Reinheckel, Santiago Rodriguez de Cordoba, Behdad Afzali, John P. Atkinson, Claudia Kemper

Immunity
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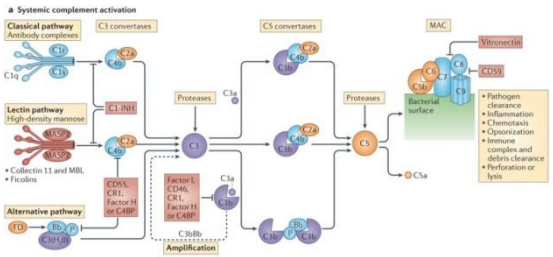
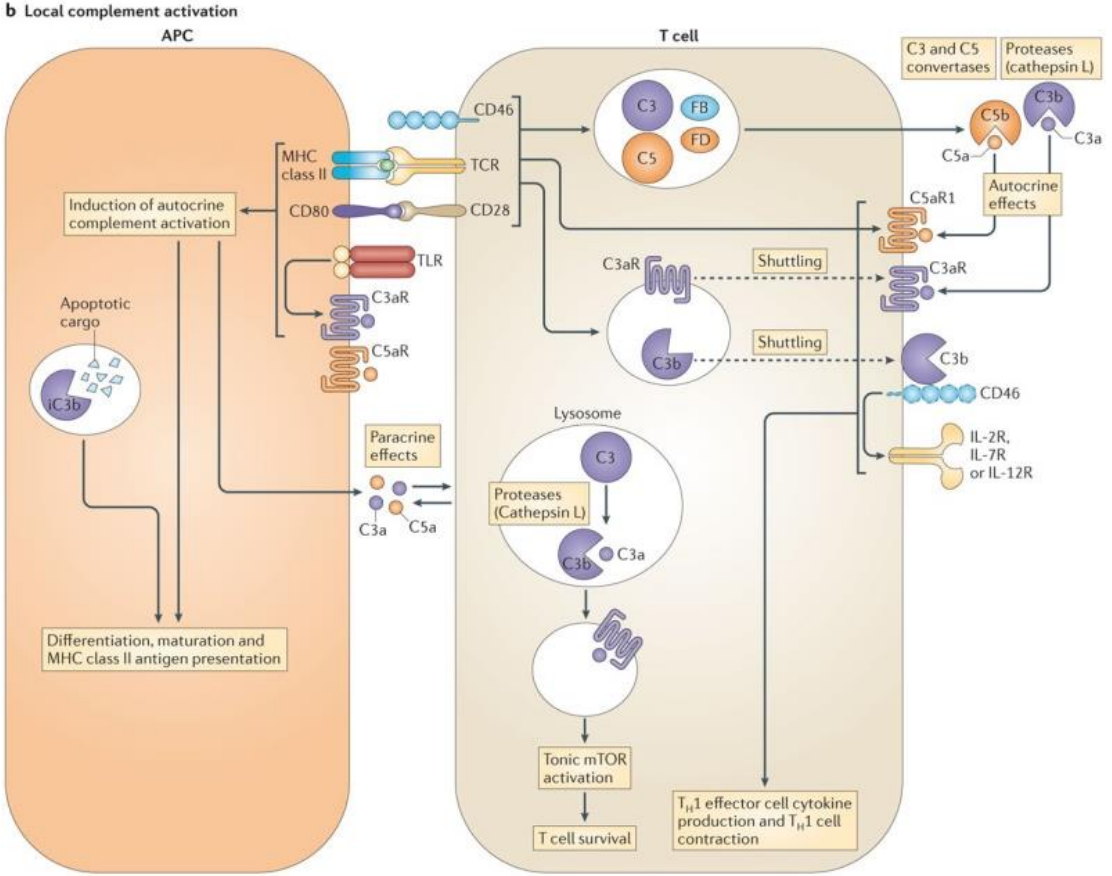
DOI: 10.1016/j.immuni.2013.10.018

CELLULAR COMPLEMENT

- Complement system is more than ‘just‘ system of serum proteins for host defence
- expressed by almost all immune cells (incl. B and T cells)
- directs both innate and adaptive immune responses
- it‘s more than a pro-inflammatory effector system
- mediates crosstalk between other cell effector systems (e.g.: growth factor receptors, metabolic sensors and the Notch system)

SYSTEMIC VS. CELLULAR COMPLEMENT





Kolev M. et al. Nature Reviews Immunology 2014

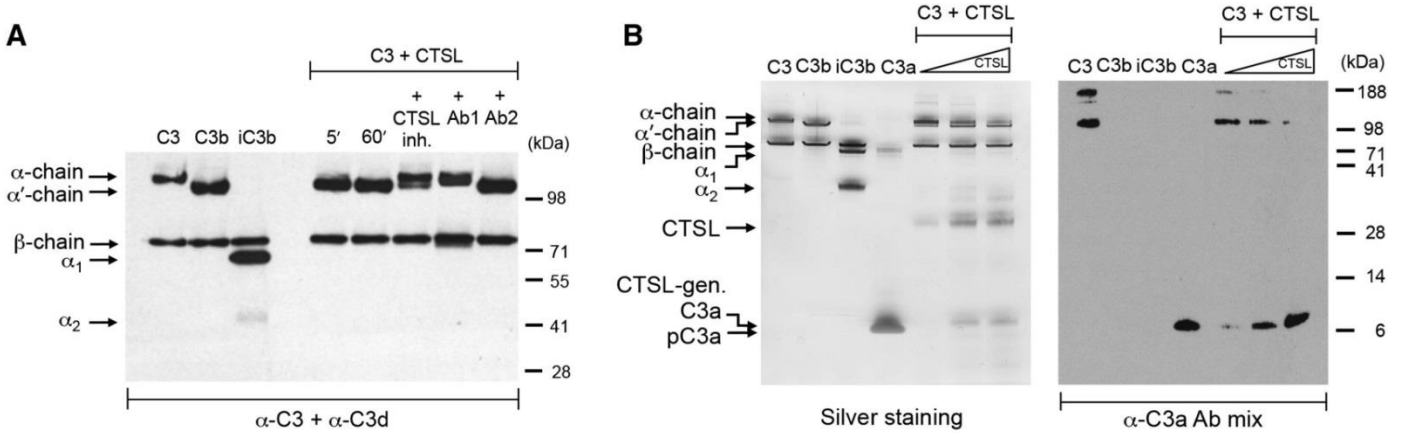


Fig. 1: Resting Human CD4⁺ T Cells Contain Stores of C3 and C3-Activating Cathepsin L

- explore potential C3 convertase-independent mechanisms > performed gene expression studies and focussed on endogenous proteases > revealed large amounts of endosomal and lysosomal proteases are regulated upon T cell activation > cathepsin B, G and L.
- they investigated if these cathepsins can cleave C3 in C3a and C3b in vitro > Fig 1A and B > CTSG and B degraded C3 only cathepsin L was able to cleave C3.
- controls for various fragments of C3 > cleavage after 5 and 60 minutes is shown and additionally no cleavage when using a cathepsin inhibitor or blocking antibody > Ab2 represents an additional control and does not block cathepsin L therefore > cleavage of C3.
- Fig 1B: generation of C3a is displayed > controls including C3a > cleavage of C3 to C3a is seen when using various concentrations of cathepsin L (50, 100, and 200 ng)

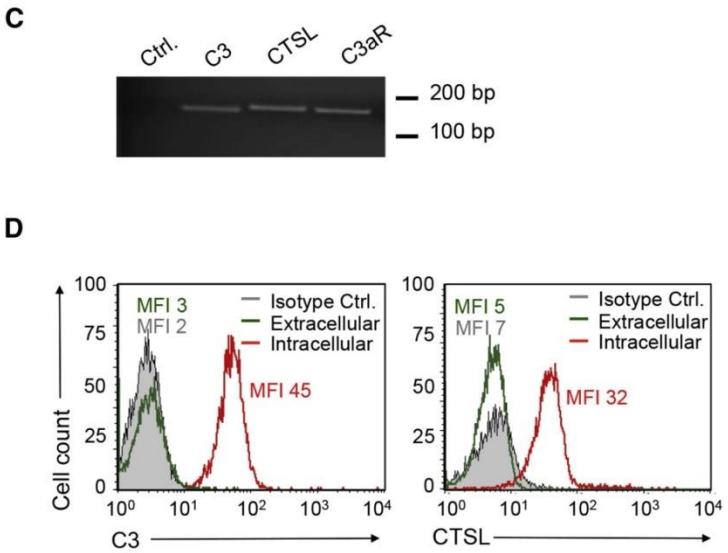


Fig. 1: Resting Human CD4⁺ T Cells Contain Stores of C3 and C3-Activating Cathepsin L

- PCR (Fig 1C) and flow cytometry (Fig 1D) performed to assess the expression of C3, cathepsin L and C3aR in CD4 T cells
- they found that in resting CD4 T cells C3 and CTSL are already expressed intracellular (red) but not extracellular (green)

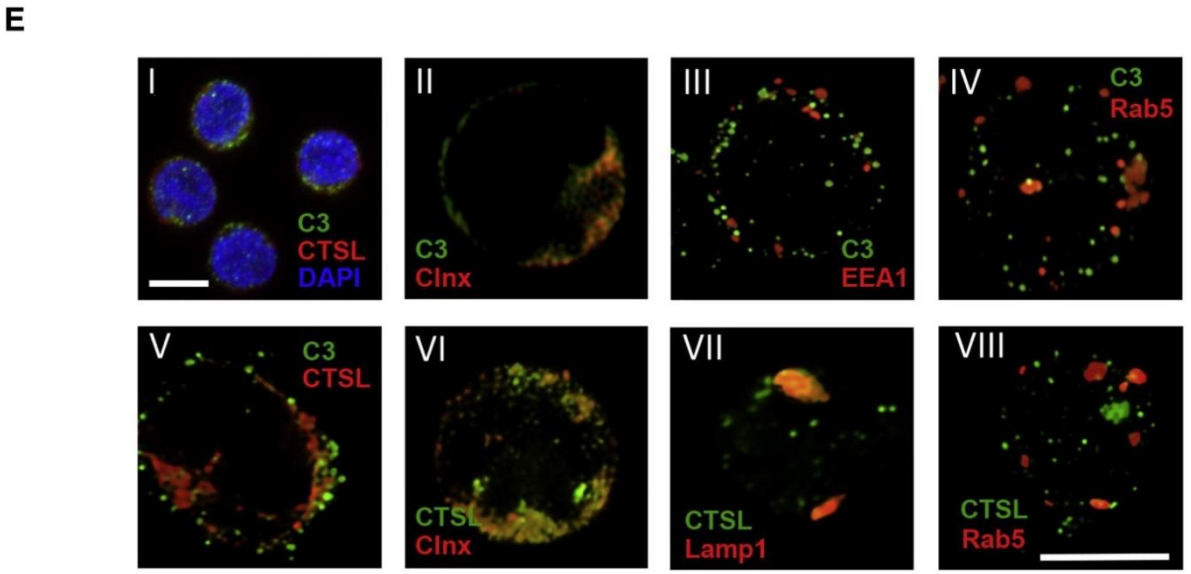
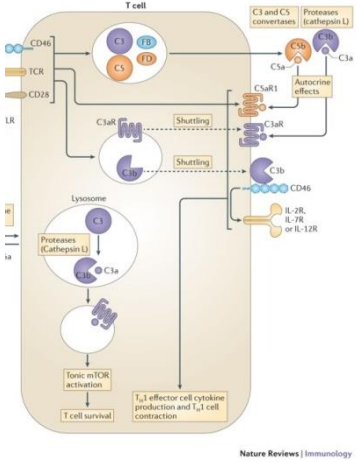


Fig. 1: Resting Human CD4$^+$ T Cells Contain Stores of C3 and C3-Activating Cathepsin L

- confocal microscopy to localize the C3 and Cathepsin L expression in T cells
- Cathepsin L was found in the ER (Clnx, calnexin), the lysosomes (Lamp1) and late-ER-derived secretory vesicles (Rab5)
- C3 also localized with to the ER (Clnx, calnexin) as well as in early and late ER-derived secretory vesicles EEA1 and Rab5)



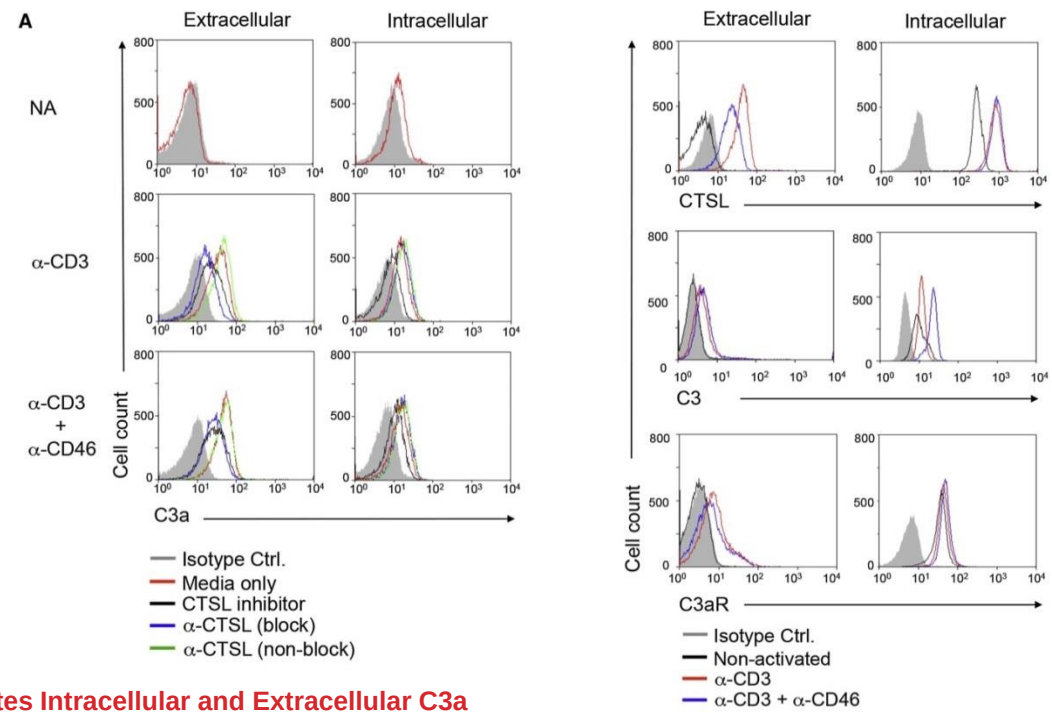


Fig. 2: CTSL Generates Intracellular and Extracellular C3a

- investigated the extracellular and intracellular presence of C3a in resting and in activated CD4 T cells > further investigate Cathepsin L-mediated processing of C3 occurs in human CD4 T cells
- For activation of the T cells > anti-CD3 and anti-CD3 and anti-CD46 antibodies for 1 hr > used antibody that specifically recognizes cleaved C3a but not C3a within the alpha chain
- on resting CD4 T cells > no C3a was detectable > but when activated with anti-CD3 (left, red line) or the combination of anti-CD3 and anti-CD46 (left, red line) > C3a appeared on the exterior of the cell
- this was significantly decreased when Cathepsin L inhibitor (left, black line) or blocking antibody (left, blue line) was added
- Cathepsin L, C3 and C3aR were also detected at the surface of the cells and inside the cells
- C3 and Cathepsin L were upregulated extracellular upon stimulation (right red and blue lines)

Cell PRESS

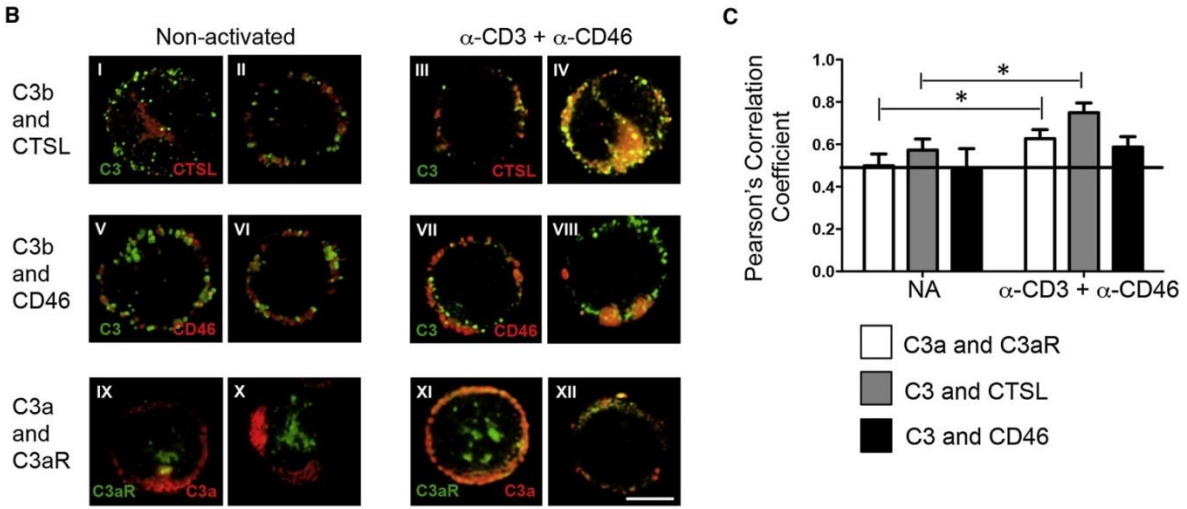


Fig. 2: CTSL Generates Intracellular and Extracellular C3a

- confocal microscopic analyses with non-activated and activated T cells > stained for C3b & Cathepsin L, C3b & CD46 as well as C3a & C3aR
- by microscopy and using the Pearson‘s Correlation coefficient > show increase of colocalization of C3a & C3aR and C3 & Cathepsin L > when T cells were activated with anti-CD3 and ant-CD46 antibodies
- they concluded that Cathepsin L generates active C3a from existing C3 pools in resting CD4 T cells and also on the surface when cells are activated

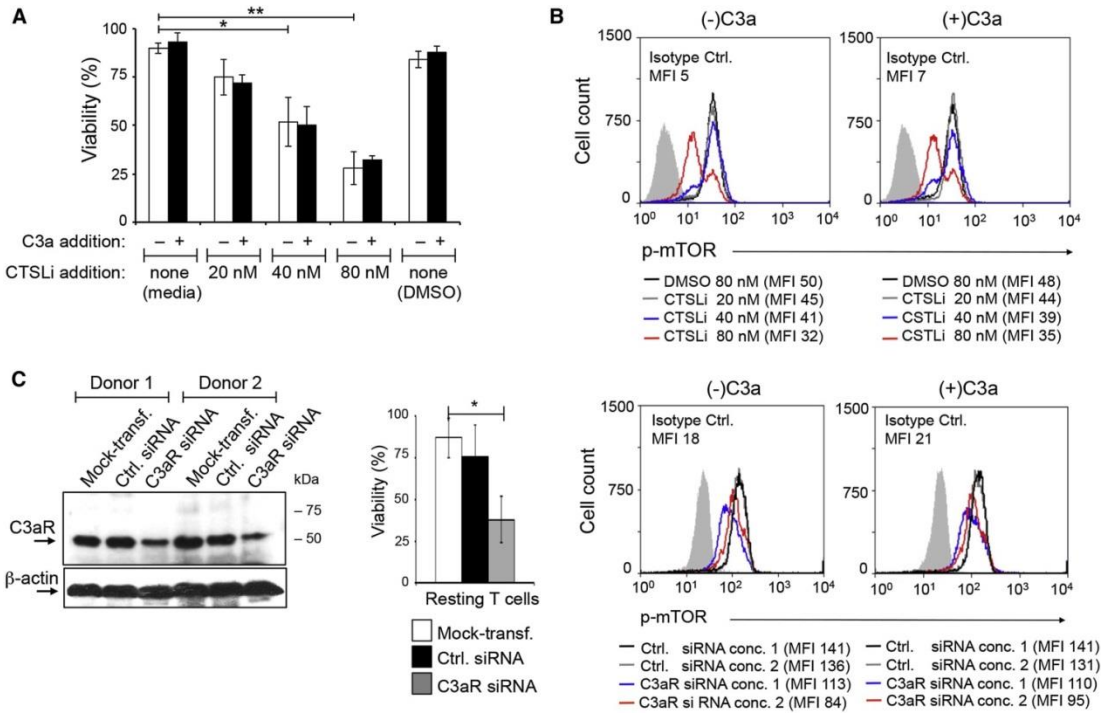


Fig. 3: CD4⁺ T Cell Survival Is Dependent on CTSL-Mediated C3 Processing and Intracellular C3aR Signaling

- during experiments using the Cathepsin L inhibitor and blockers > they noticed that cells entered an apoptotic state within 8-12 hrs > which can be seen in Fig 3A > increasing amounts of inhibitor > cell viability goes down with or without addition of C3a
- Since activation via T cell receptors is connected with mTor activation > required for T cell survival and induction of T cell responses > investigated phosphorylation of mTor
- they found > in line with the viability assays > Cathepsin L inhibitors decreased phosphorylation of mTor (Fig.3B upper panel, left) and addition of C3a (Fig.3B upper panel, right) could not rescue the cells
- they incubated the T cells with various concentrations of C3aR siRNAs > also found phosphorylation of mTor was reduced and also no rescue effect with addition of C3a. (Fig 3C and 3B lower panel)

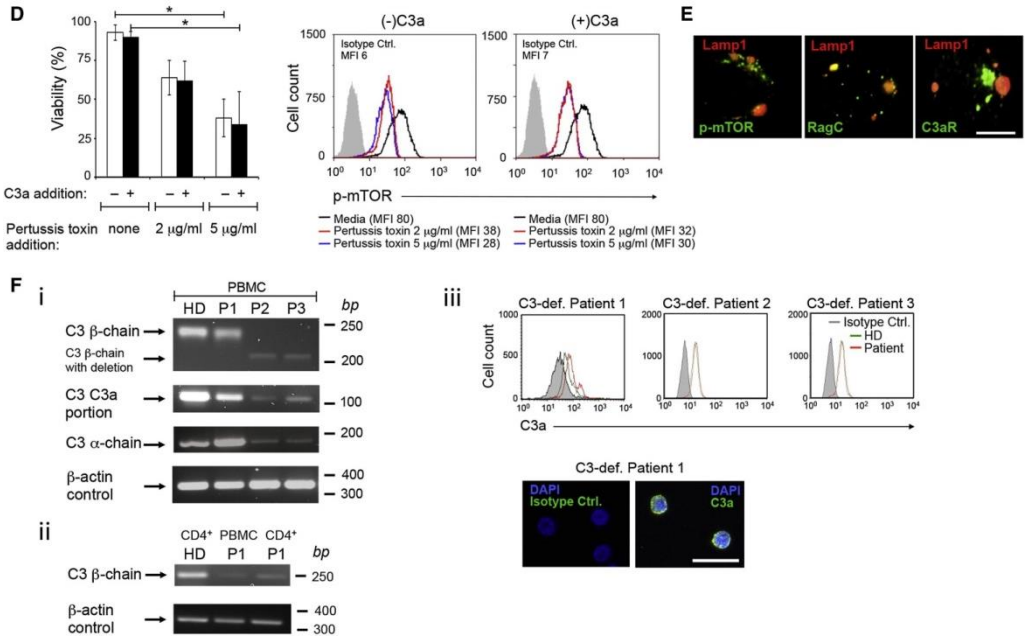


Fig. 3: CD4⁺ T Cell Survival Is Dependent on CTSL-Mediated C3 Processing and Intracellular C3aR Signaling

- same results > viability and phosphorylation of mTor were detected > using the inhibitor for G protein–coupled receptors > pertussis toxin
- supplementary figures show > this inhibitor is not toxic but demonstrates specific effect in CD4 T cells
- Fig.3E shows that C3aR expression is also detected in lysosomes of resting T cells, but resting cells do not express C3aR on the surface
- in C3 deficient patient > cannot produce IFNg > T cells still survive and proliferate normally > Why?
- C3 not produced in the liver > but cells of patients produce C3 > PBMCs isolated from 3 patients > able to produce C3 in comparable amount as healthy > shown by PCR
- panel below (II) shows PCR result from PBMC and CD4 cells from patient 1
- C3a expression of patients and healthy donor is shown > equal protein expression of C3a by FACS analyses and confocal microscopy > indicates cellular and not systemic generation of C3a.

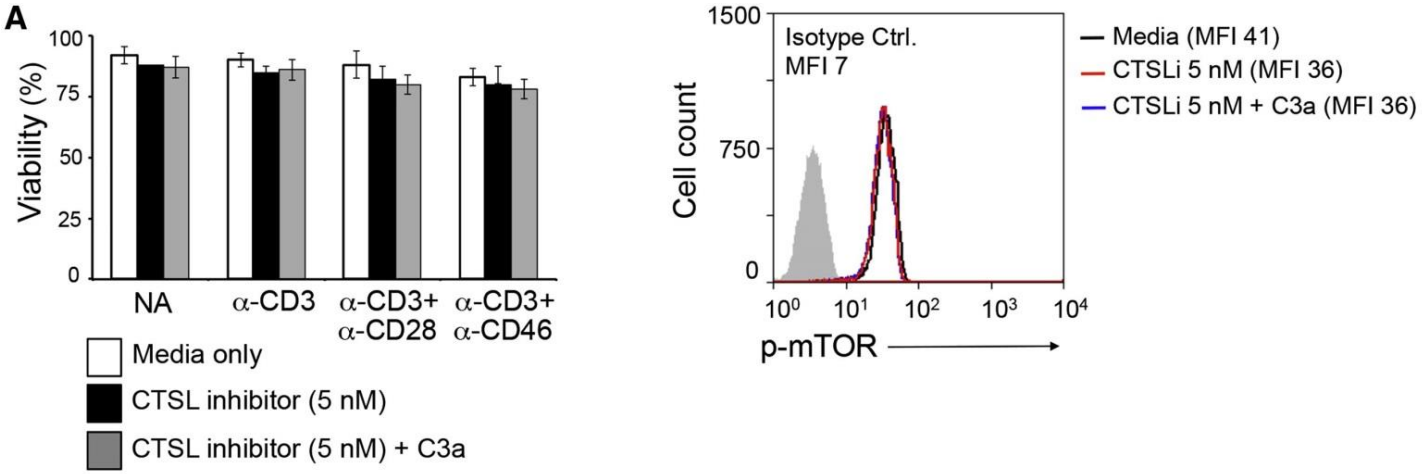


Fig. 4: Th1 and Th17 Cell Induction Requires Cell Surface Activation of CD46 and C3aR by CTSL-Generated C3 Activation Fragments

- Cells were activated > treated with low level Cathepsin L inhibitor > no killing of cells > therefore no affect on cell viability or mTor activation

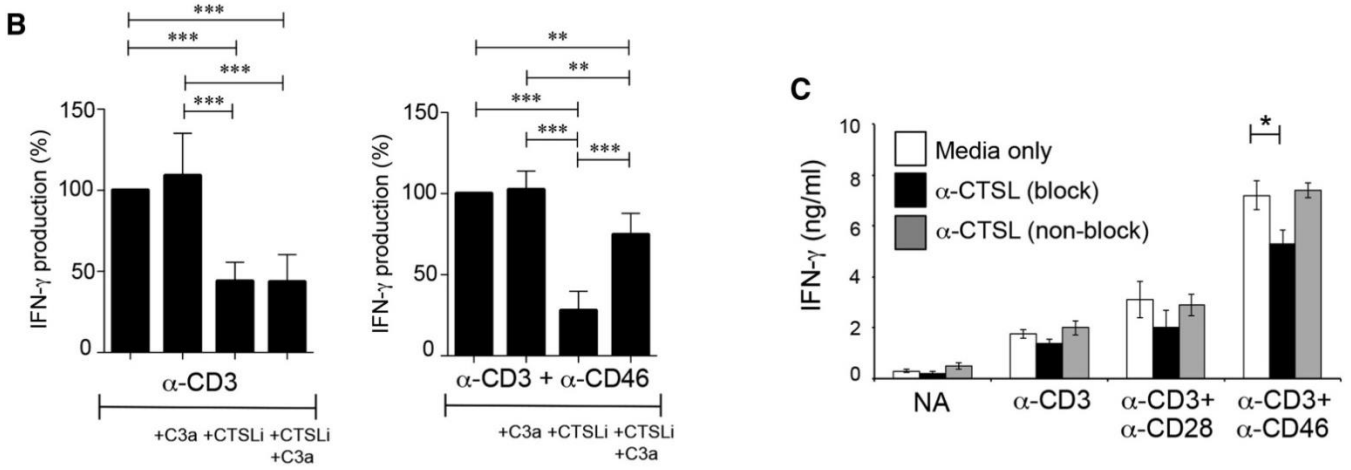


Fig. 4: Th1 and Th17 Cell Induction Requires Cell Surface Activation of CD46 and C3aR by CTSL-Generated C3 Activation Fragments

- already at this low level of inhibition > IFNg secretion was reduced by 50% > could be restored when C3a was added
- restoring only worked > cells were activation or stimulated with both anti-CD3 and anti-CD46 antibody but not with anti-CD3 only > anti-CD46 mimics C3b generation and binding
- Same results were found > using the Cathepsin L blocking antibody > no effect using unspecific antibody

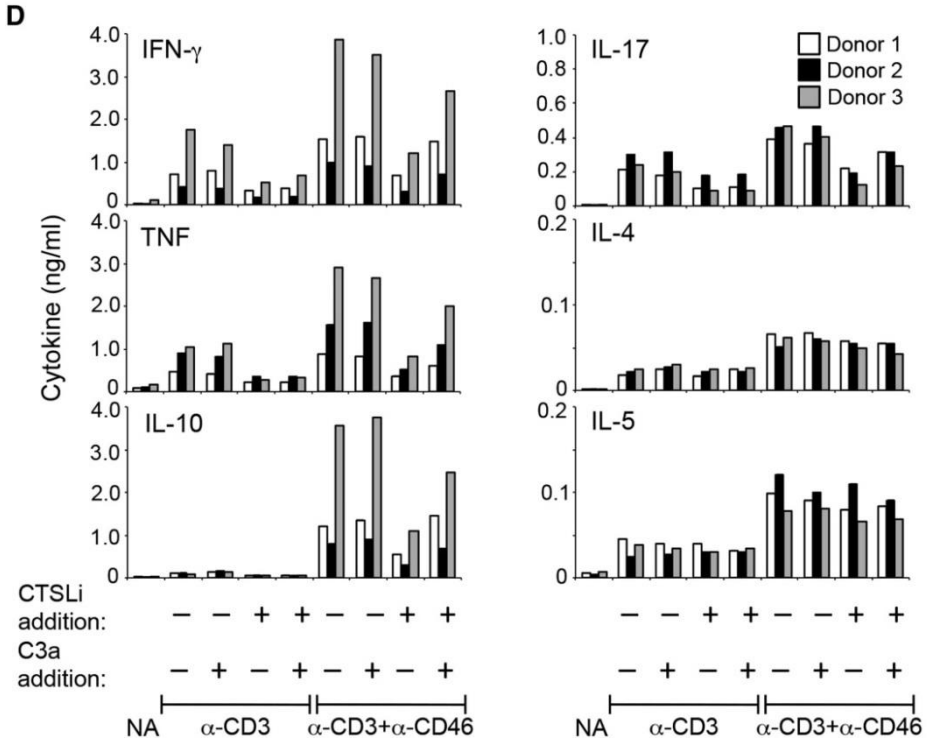


Fig. 4: Th1 and Th17 Cell Induction Requires Cell Surface Activation of CD46 and C3aR by CTSL-Generated C3 Activation Fragments

- investigation of cytokine secretion by cytometric bead arrays > Th1 and Th17 related cytokines were only induced when both signals – CD3 and CD46 – were present > Th2 related cytokines were barely affected
- Addition of Cathepsin L inhibitor > decreased cytokine secretion and could be restored upon C3a administration
- mouse experiments could not verify results > leading to the conclusion that cleavage of C3 works in an Cathepsin L independent manner

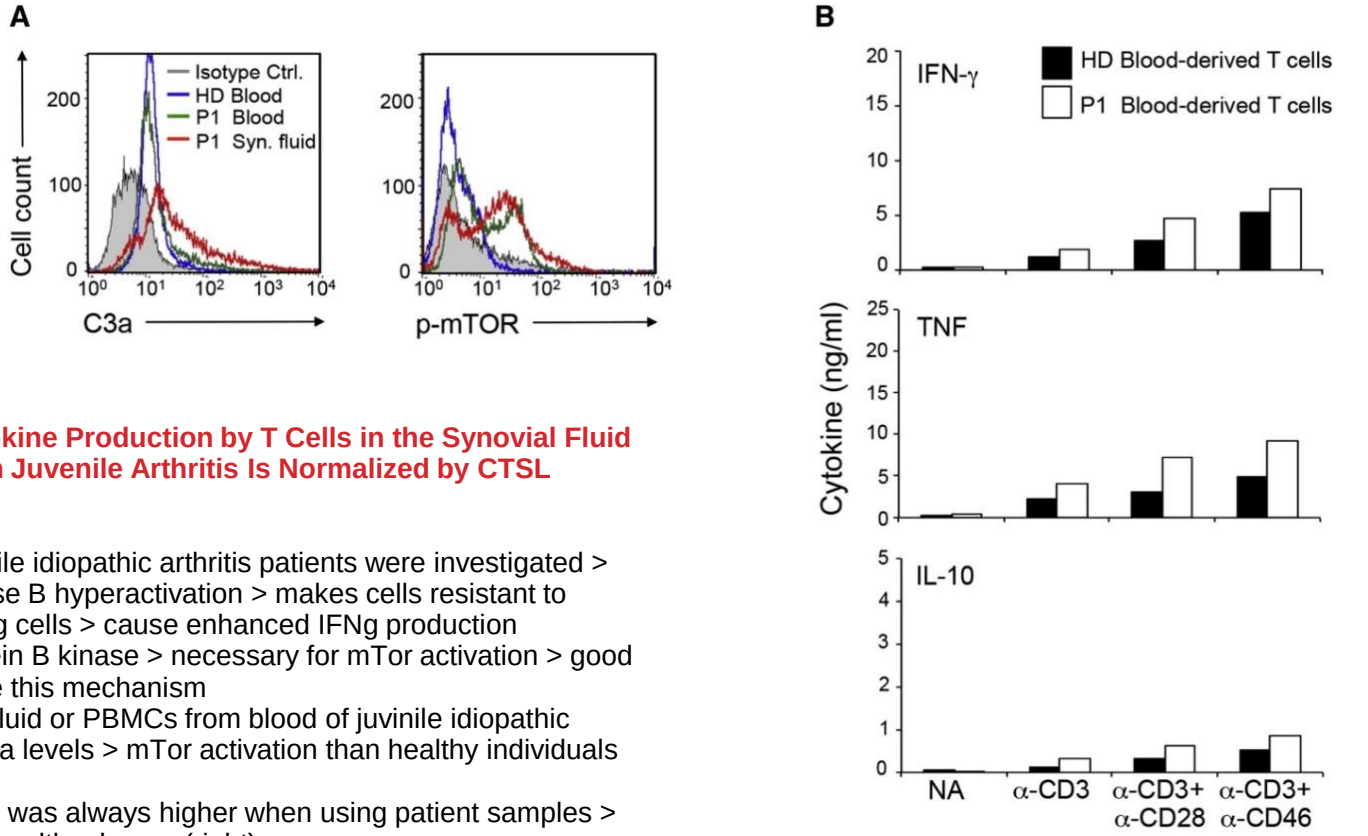


Fig. 5: Enhanced Cytokine Production by T Cells in the Synovial Fluid from a Patient with Juvenile Arthritis Is Normalized by CTSL Inhibition

- samples from juvenile idiopathic arthritis patients were investigated > have a protein kinase B hyperactivation > makes cells resistant to suppression by Treg cells > cause enhanced IFNg production
- C3aR induces protein B kinase > necessary for mTor activation > good model to investigate this mechanism
- cells from synovial fluid or PBMCs from blood of juvinile idiopathic arthritis > higher C3a levels > mTor activation than healthy individuals (left)
- cytokine expression was always higher when using patient samples > than samples from healthy donors (right)

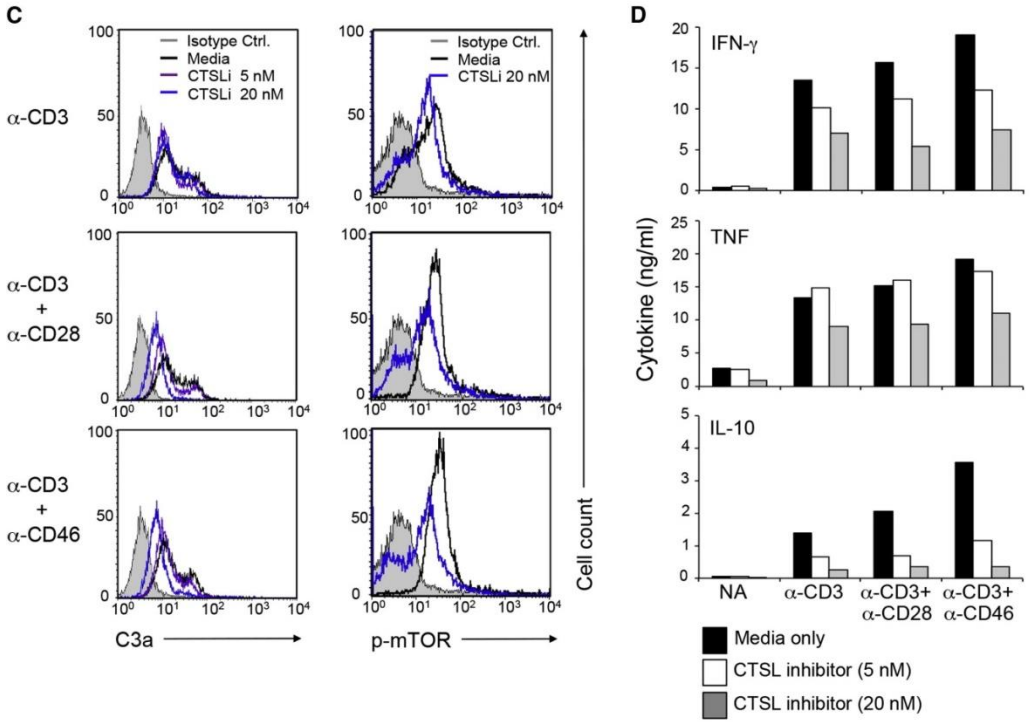


Fig. 5: Enhanced Cytokine Production by T Cells in the Synovial Fluid from a Patient with Juvenile Arthritis Is Normalized by CTSL Inhibition

- further investigated patient samples > added Cathepsin L inhibitor to the experimental setup > found that C3a expression and mTor (left) activation as well as IFNg and TNF induction (right) could be normalized in a dose-dependant manner > using the inhibitor

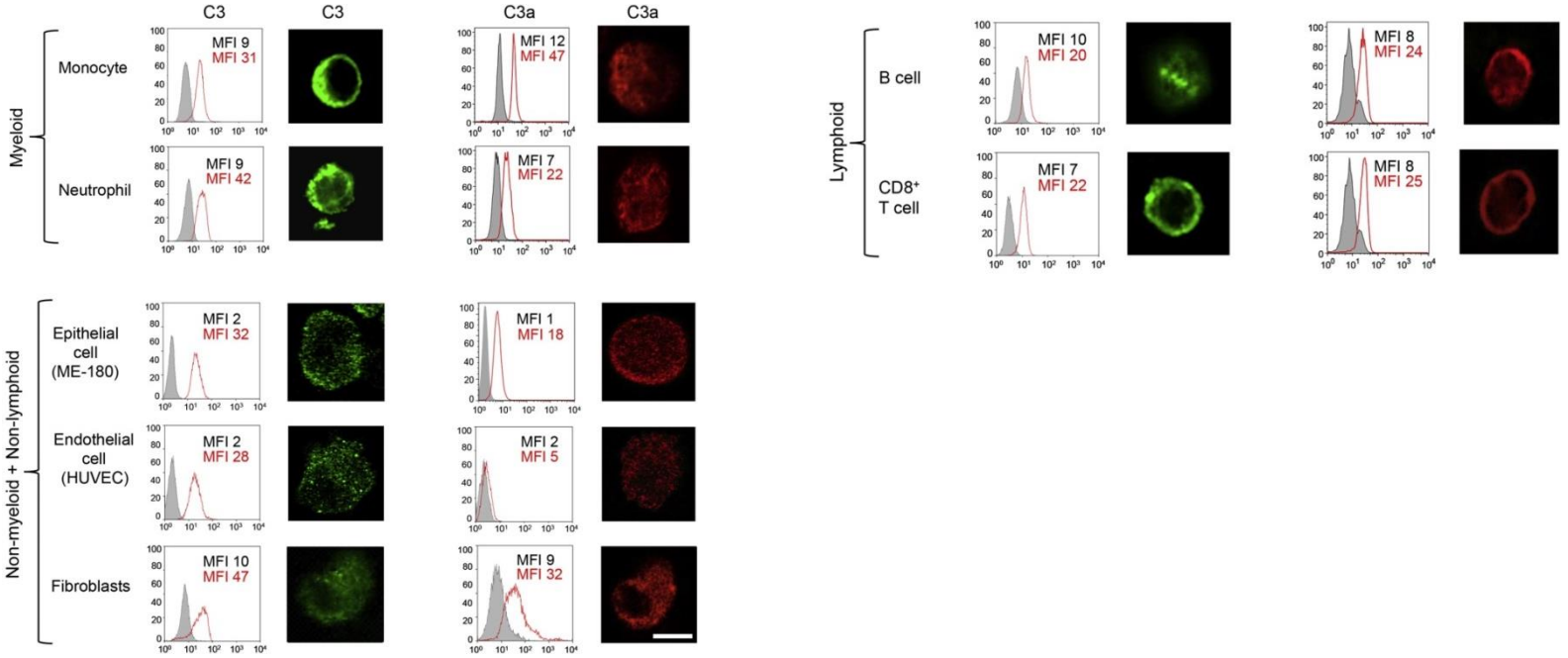
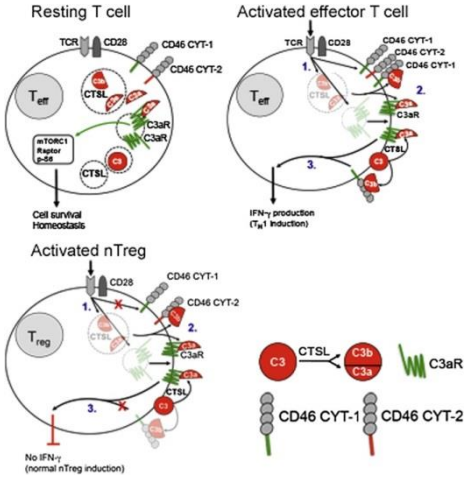


Fig. 7: Intracellular C3 Stores and "Tonic" Intracellular C3a Generation Occurs in Myeloid, Lymphoid, and Nonmyeloid, Nonlymphoid Cell Populations

- showed that intracellular C3 activation is not a T cell specific phenomenon > a general mechanisam
- a newly discovered intracellular pathway that regulates cell activation, immune regulation, maintenance and homeostasis.

Conclusions

- cellular C3 cleavage is C3 convertase independant > T-cell expressed Cathepsin L cleaves C3
- resting T cells contain an intracellular pool of Catheps > fast cleavage of C3 upon stimulation
- C3a engages with C3aR > C3aR is expressed on lysosomes > on the surface when T cells are activated
- this systems activates mTor > better cell survival
- translocation of C3aR to the surface in effector cells > activation of Th1 cell-mediated responses
- Treg cells lack CD46-CYP-1 upregulation > no IFNg
- differences between mice and men > not Cathepsin dependant
- data obtained represents a shift in our thinking of complement – not only as a part of the innate immune systems that opsonizes and lyses pathogens as well as induction of pro-inflammatory cytokines
- but also a intracellular regulator of cell activation, survival and other cell effector systems.



THANKS for your ATTENTION!!!