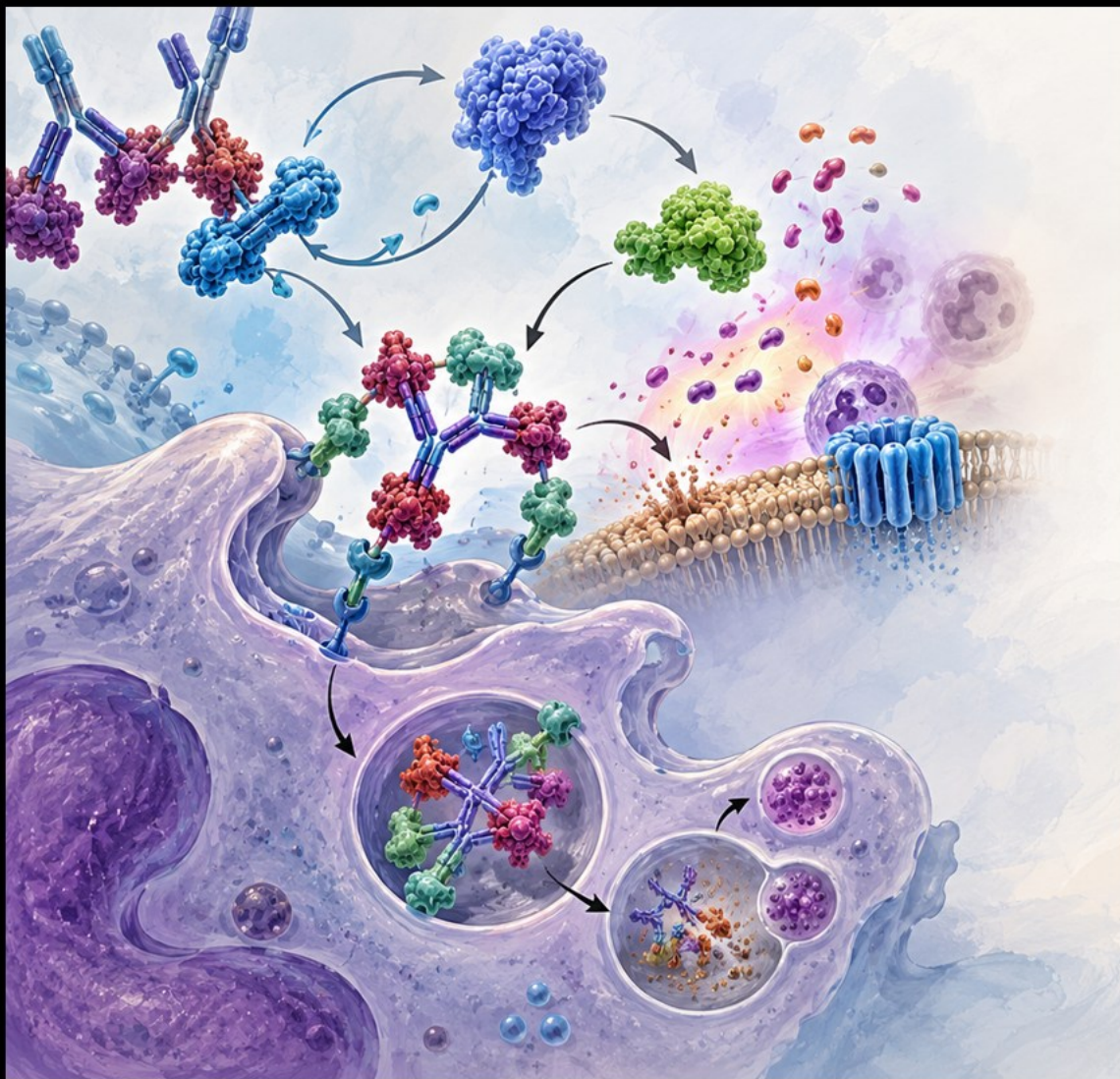




Σύστημα Συμπληρώματος

Αναστάσιος Καραμανάκος

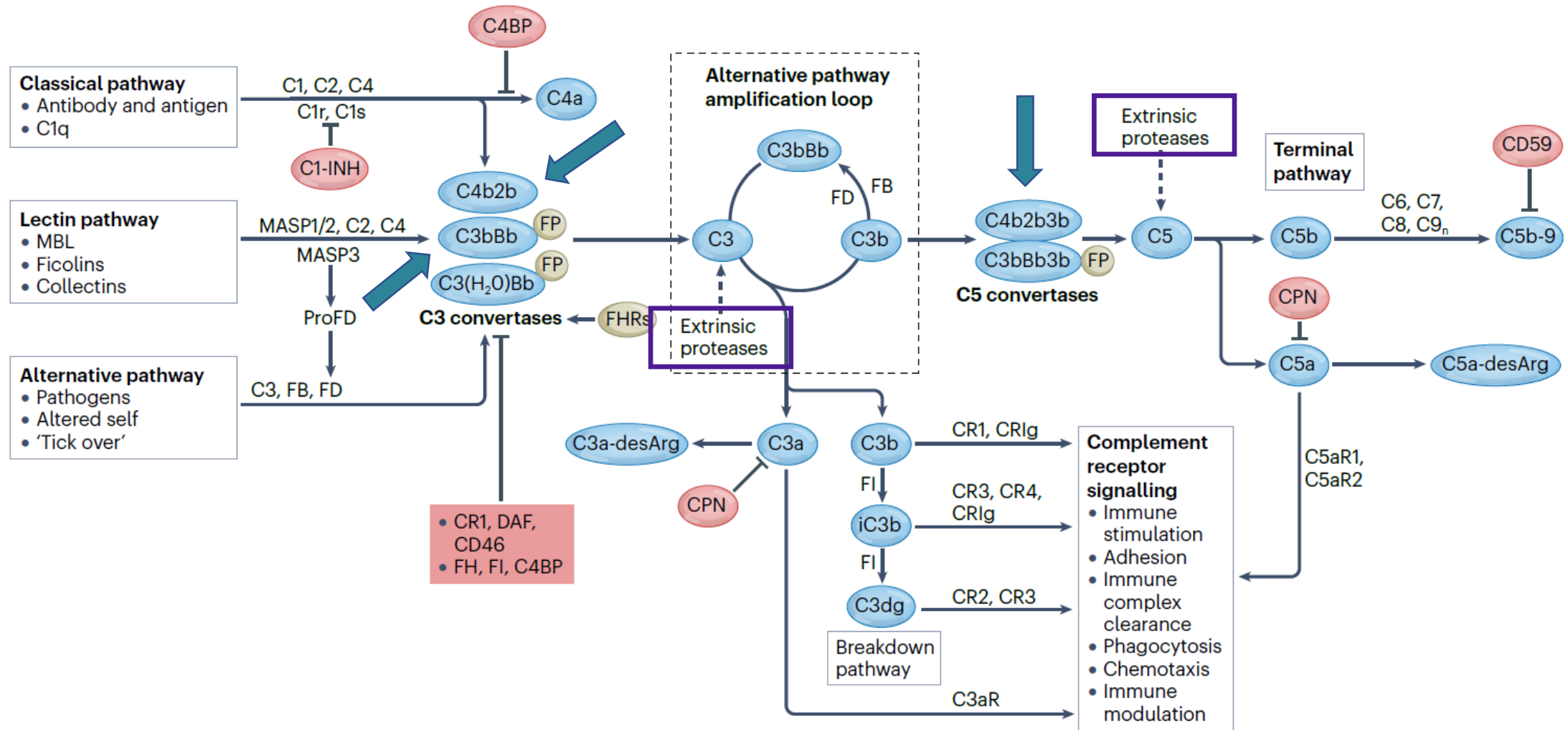
Ρευματολόγος

ΓΝ «Ο Ευαγγελισμός»

Σύγκρουση Συμφερόντων

☐ Καμία για τη συγκεκριμένη ομιλία

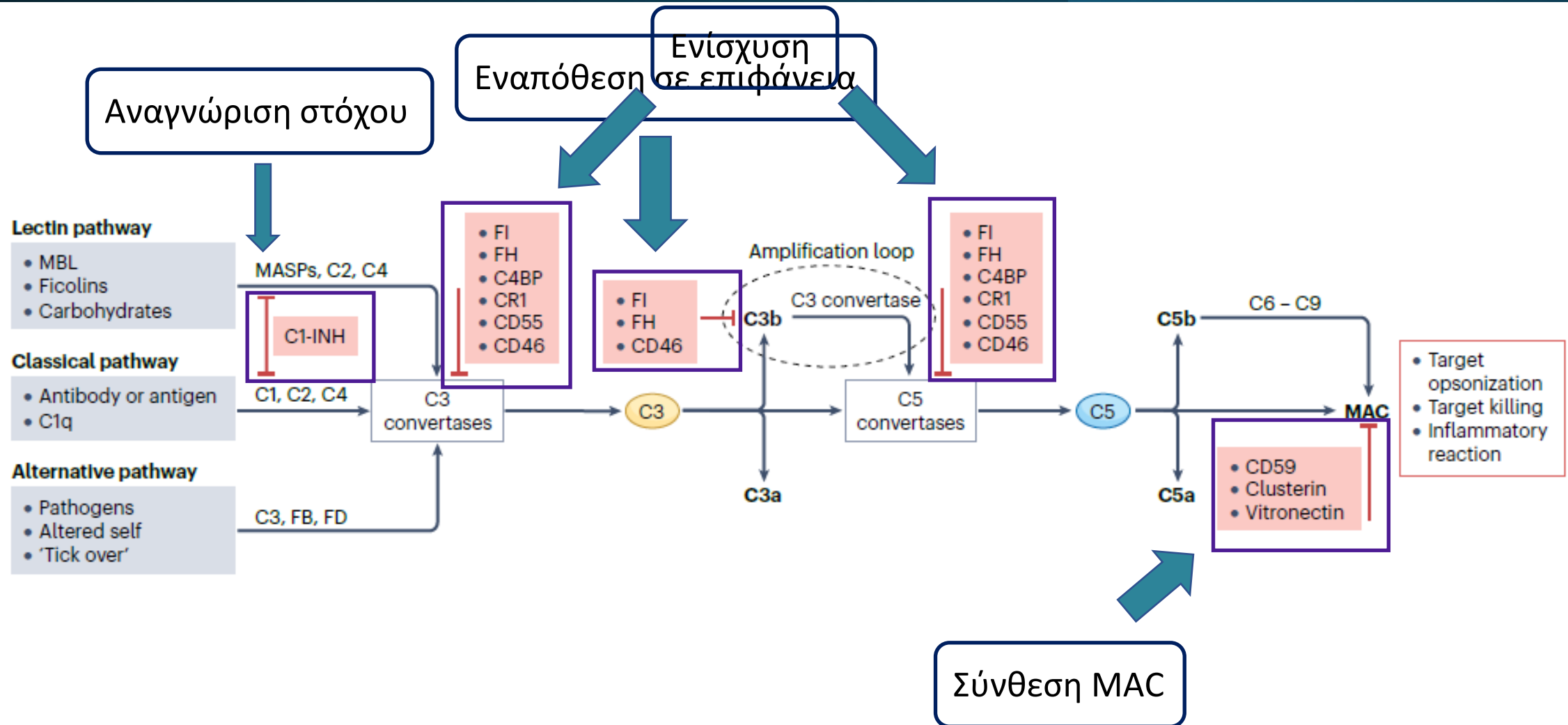
Σύστημα Συμπληρώματος (ΣΣ): “Canonical” & “Non – Canonical Ενεργοποίηση



Σύστημα Συμπληρώματος: Μόρια Ενεργοποίησης και Μηχανισμοί

Classical pathway	Alternative pathway	Lectin pathway
Immune complexes (IgM/IgG)	‘Tickover’	G0 carbohydrate glycoforms
C-reactive protein	Amplification loop	Cytokeratin-1
Apoptotic bodies (C1q)	IgA immune complexes	Acetylated proteins
Beta-amyloid fibrils	Endotoxin	IgG4 pathogenic glycoforms
Serum amyloid P	Polysaccharides	Mannose, N-acetylglucosamine, fucose
Mitochondrial products	C3 nephritic factor	
C4 nephritic factor	Cobra venom factor	
PTX-3	Properdin	
Coagulation system ‘cross-talk’		

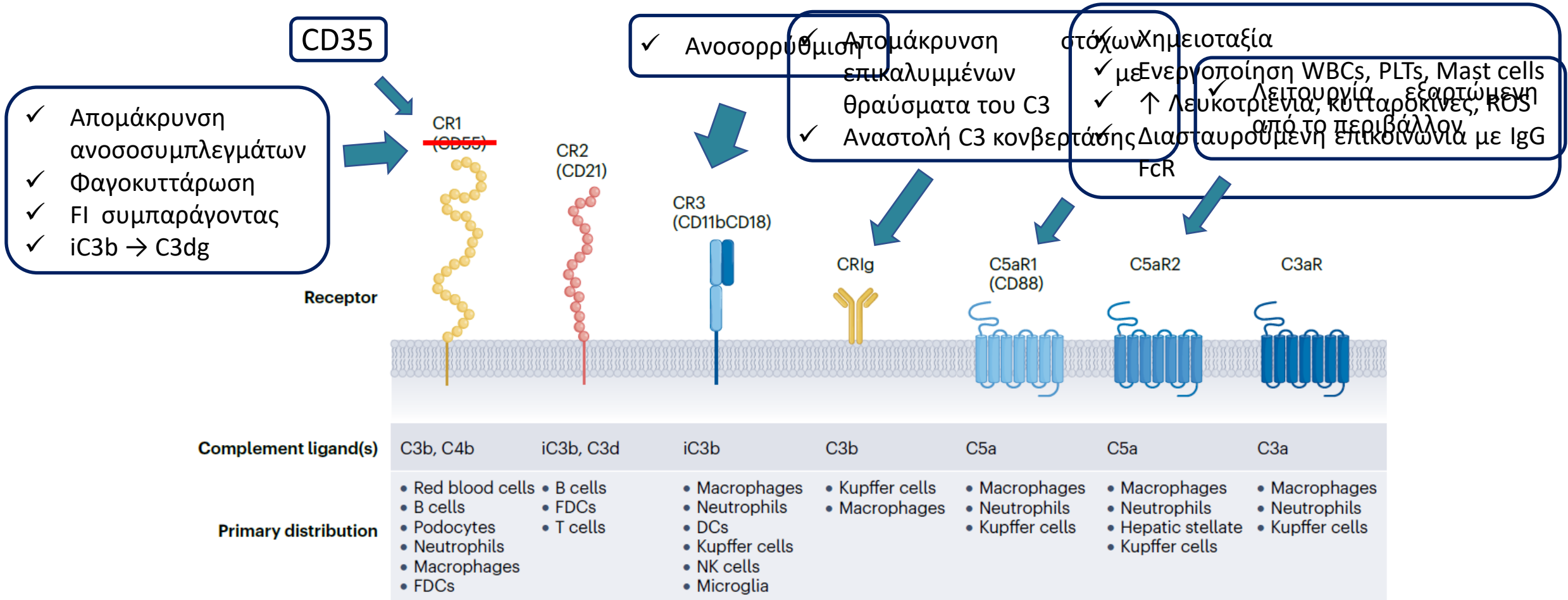
Σημεία Ρύθμισης και Ελέγχου του Συστήματος του Συμπληρώματος



Ρυθμιστικά Συστήματα του Συμπληρώματος

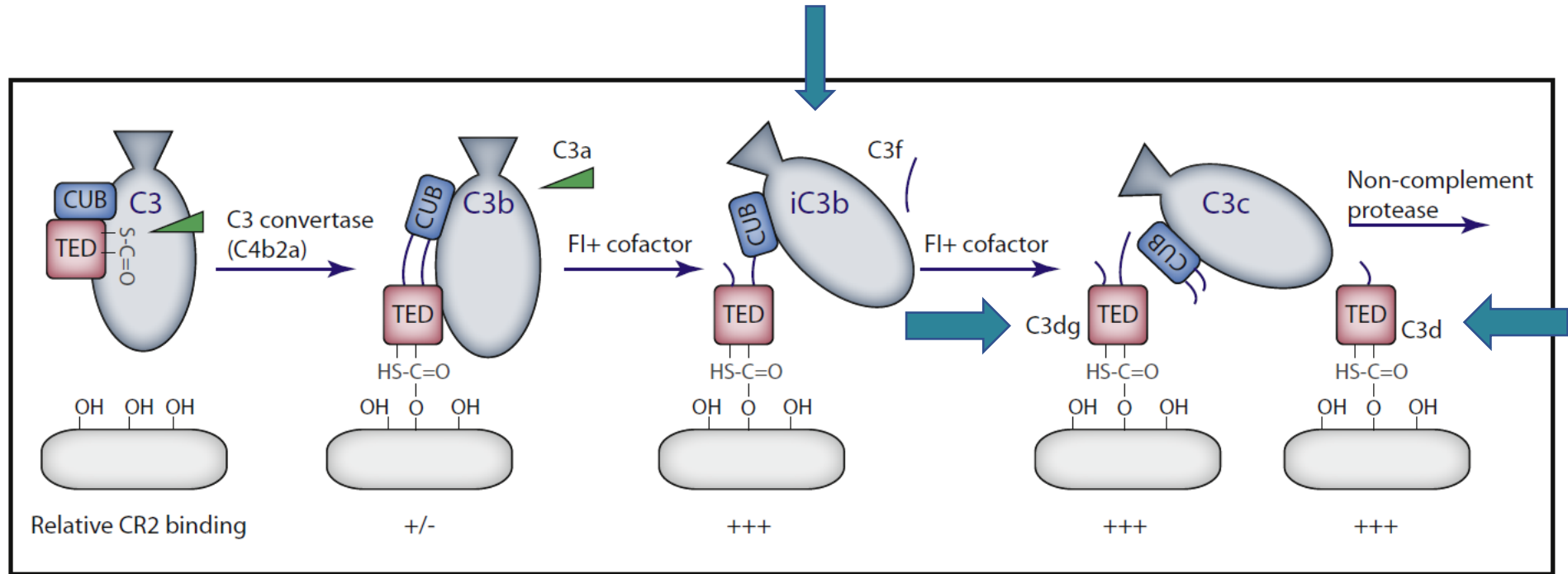
Regulator	Alternate name(s)	Localisation of activity	Molecular target(s) of inhibitory activity	Mechanism of action	Primary clinical manifestation with mutations or polymorphic/expression variation
C1-INH	n/a	Fluid phase	C1r/C1s MASP-1/MASP-2	Protease trap	HAE
Factor H	FH	Fluid phase Cell surface Matrix	C3b C3/C5 alternative pathway convertases	Decay-acceleration Cofactor activity	aHUS AMD C3 glomerulopathy (C3G) Infection
MAp44	n/a	Fluid phase	MASP-1	Competitive inhibitor	Infection (associated with MASP-1 deficiency)
MAp19	n/a	Fluid phase	MASP-2	Competitive inhibitor	Infection (associated with MASP-2 deficiency)
C4b-binding protein	C4-bp	Fluid phase	C3/C5 classical pathway convertases	Decay-acceleration Cofactor activity	aHUS
Factor I	FI	Fluid phase Cell membrane Matrix	C3b iC3b C4b iC4b	Protease (requires cofactor)	Recurrent bacterial infections (low C3) aHUS
Decay-accelerating factor/CD55	DAF CD55	Intrinsic membrane	C3/C5 pathway convertases	Convertase decay-acceleration	PNH CHAPLE disease
Membrane cofactor protein	MCP CD46	Intrinsic membrane	C3b C4b	Cofactor for Factor I	aHUS
Membrane inhibitor of reactive lysis	MIRL CD59	Intrinsic membrane	C8 C9	Disruption of C8:C9 and poly-C9 binding interactions	PNH Haemolysis with neuropathy
Vitronectin	VTN	Fluid phase	MAC	Disruption of binding interactions	NA
Clusterin	CLU	Fluid phase	MAC	Disruption of binding interactions	NA
Properdin	P	Fluid phase Cell membrane Matrix	C3/C5 convertases	Stabilises alternative pathway C3/C5 convertases	Infection
Complement factor H-related 1	CFHR1	C3 activation fragments Cell membrane Matrix Apoptotic cells CRP	FH function Potentially immune response	Dysregulates complement	AMD aHUS Anti-FH autoantibodies
Complement factor H-related 2	CFHR2	C3 activation fragments Cell membrane Matrix	FH function Potentially immune response	Dysregulates complement	C3G
Complement factor H-related 3	CFHR3	C3 activation fragments Cell membrane Matrix	FH function Potentially immune response	Dysregulates complement	aHUS Anti-FH autoantibodies
Complement factor H-related 4	CFHR4	C3 activation fragments Cell membrane Matrix CRP	FH function potentially immune response	Dysregulates complement	Infection
Complement factor H-related 5	CFHR5	C3 activation fragments Cell membrane Matrix Apoptotic cells CRP Properdin	FH function potentially immune response	Dysregulates complement	C3G

Υποδοχείς του Συστήματος του Συμπληρώματος, Κυτταρική Κατανομή και Βασικές Λειτουργίες



CR1g: Complement Receptor of the Immunoglobulin superfamily

Ομοιοπολική Σύνδεση του Αντιγόνου με το C3b και η διάσπαση του σε Ισοδύναμους Συνδέτες του CR2



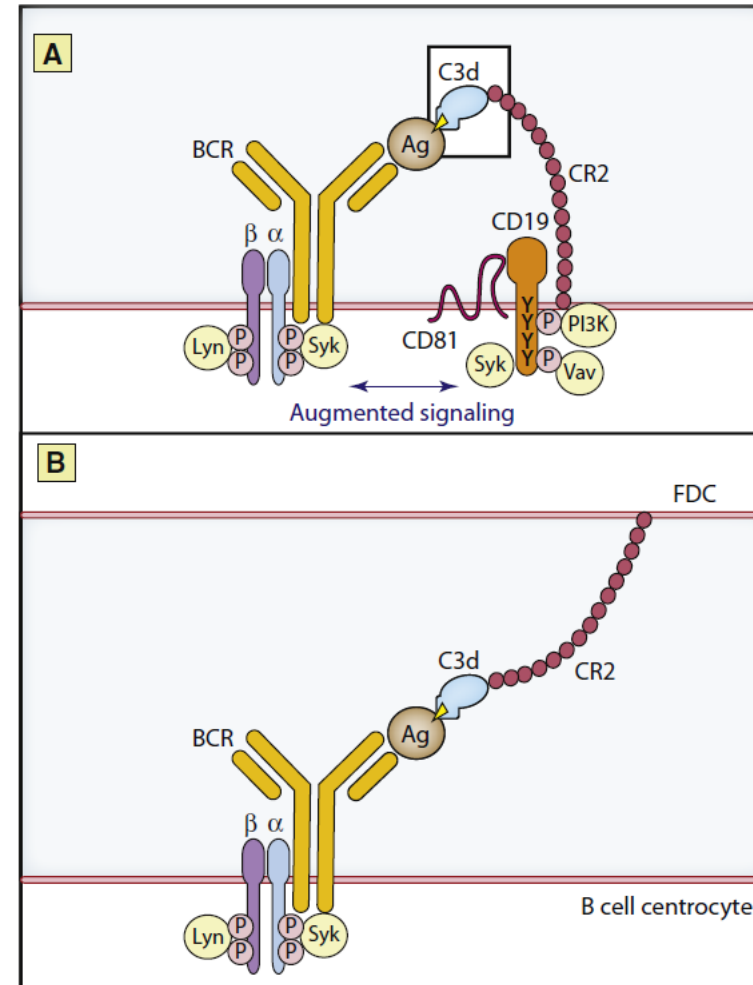
CUB = complement C1r/C1s, Uegf, Bmp1 domain

Το Σύστημα του Συμπληρώματος ως Γέφυρα Σύνδεσης μεταξύ της Εμφυτης και της Επίκτητης Ανοσίας

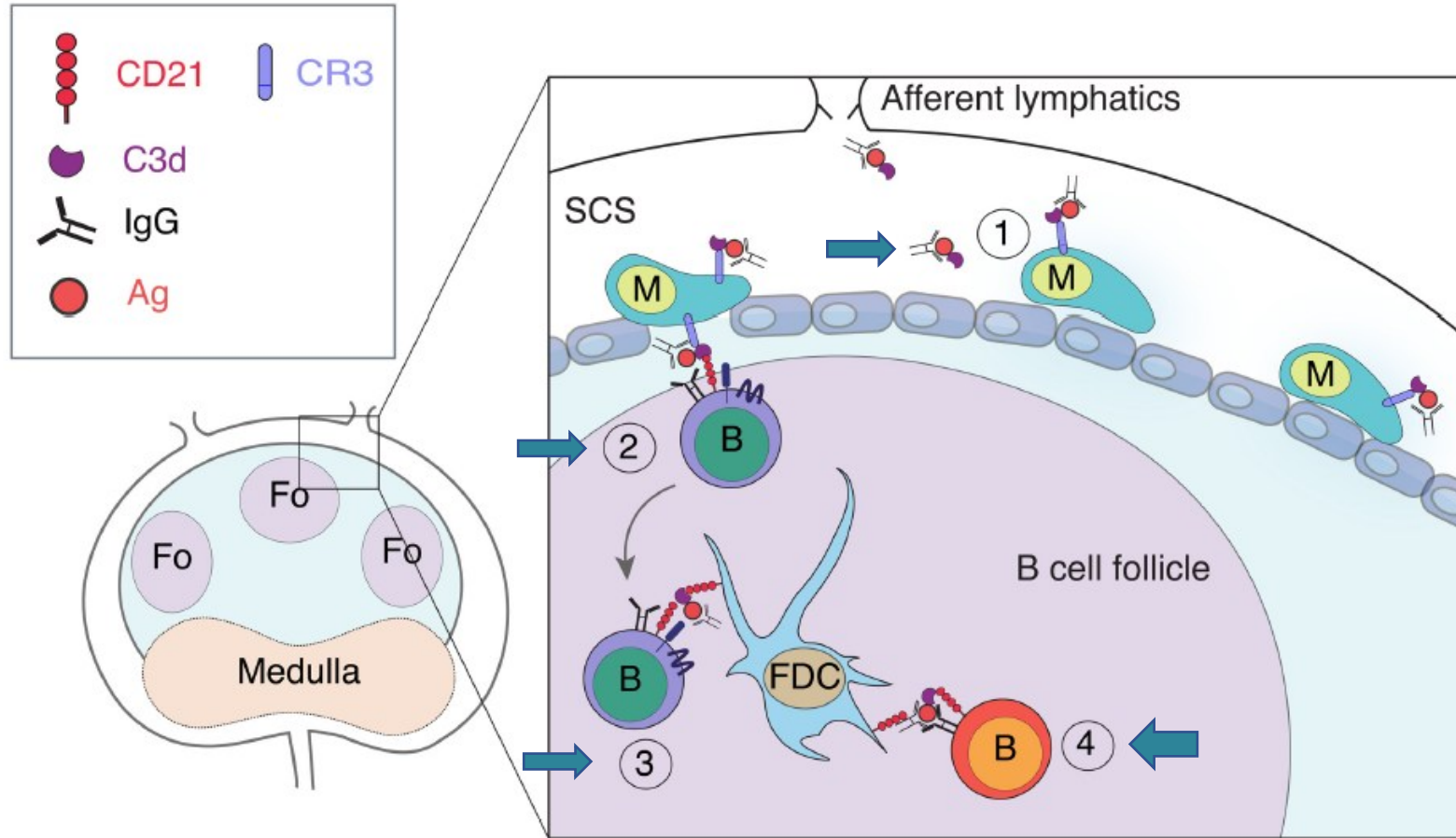
- A. Συνδέσμευση του υποδοχέα των Β λεμφοκυττάρων (BCR) με τον υποδοχέα CR2
- B. Μοριακός ανοσοενισχυτικός ρόλος του C3d

❑ C3d-binding - CR2:

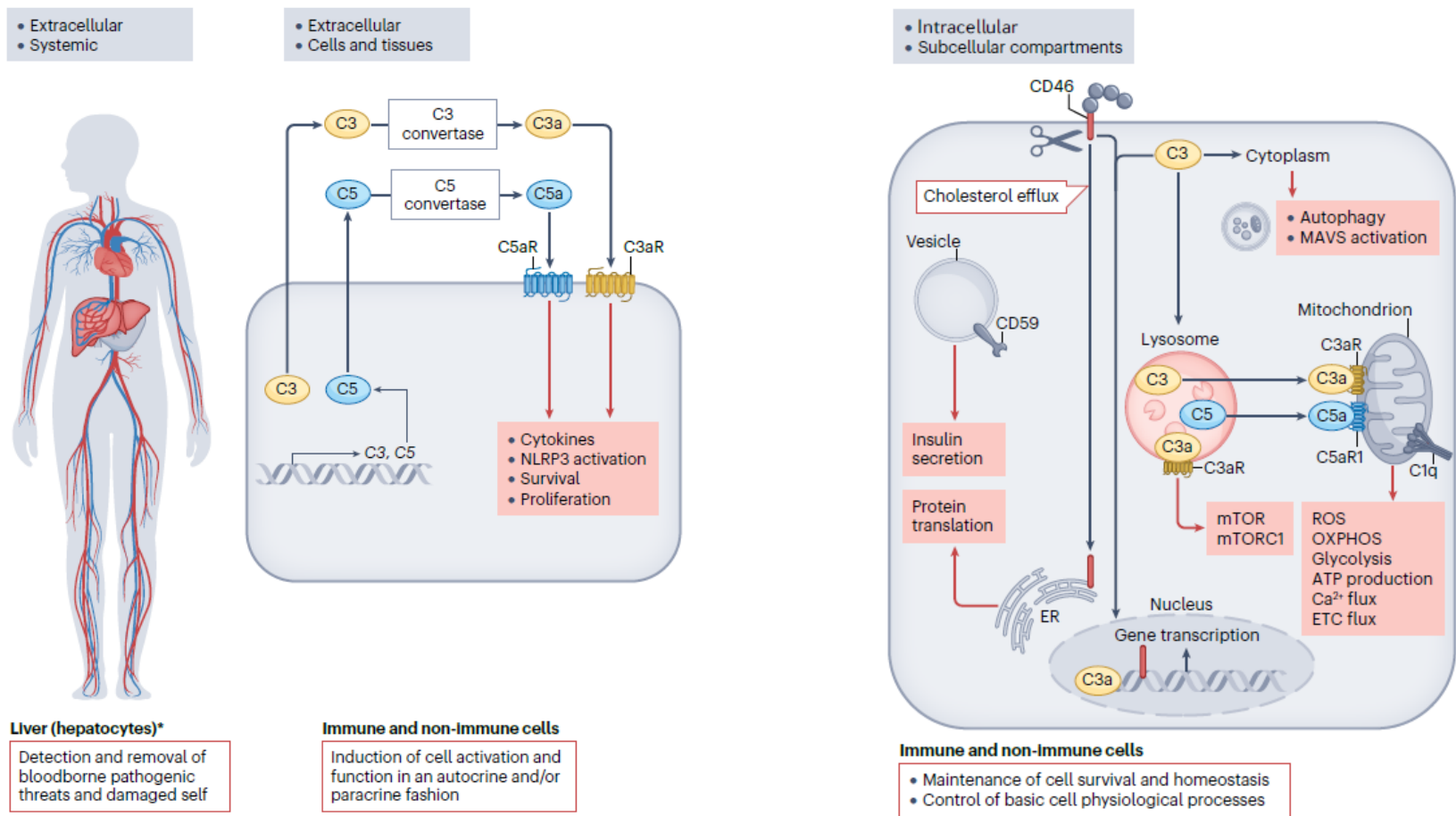
- A. Συν – διεγερτικό μόριο για τα Β – κύτταρα
- B. ↓ το κατώφλι ενεργοποίησης των Β – κυττάρων



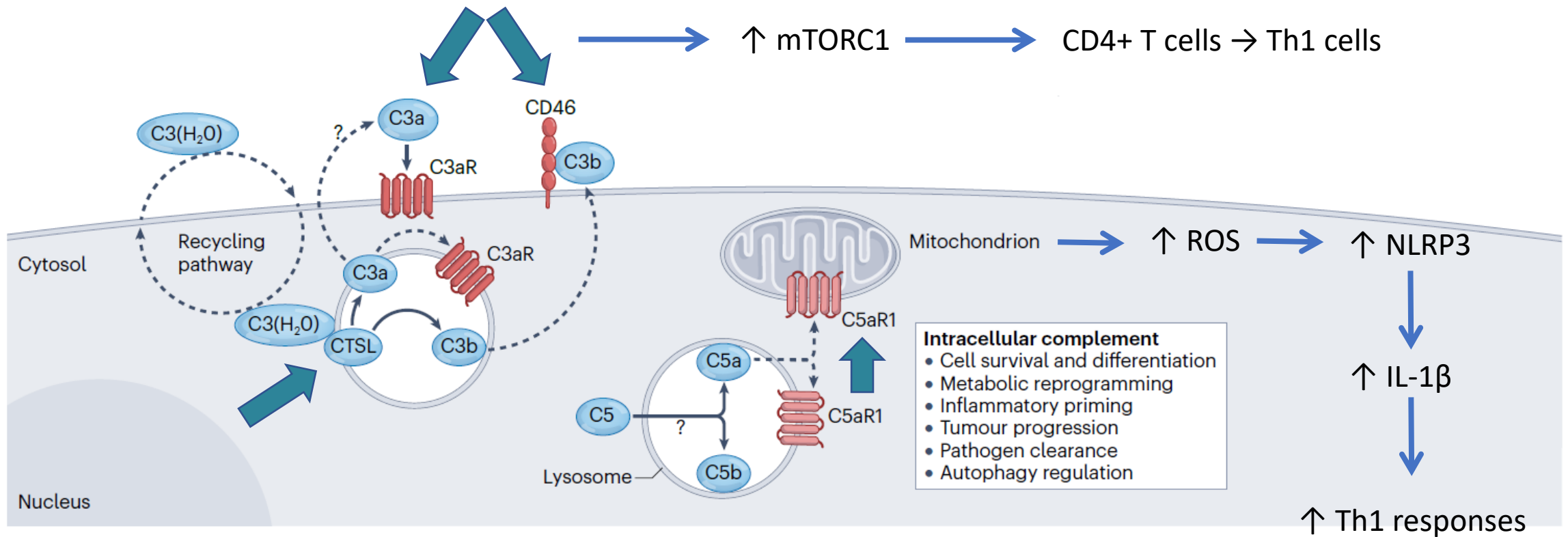
Οδοί Αναγνώρισης Αντιγόνου από τα Β λεμφοκύτταρα στον Λεμφαδένα



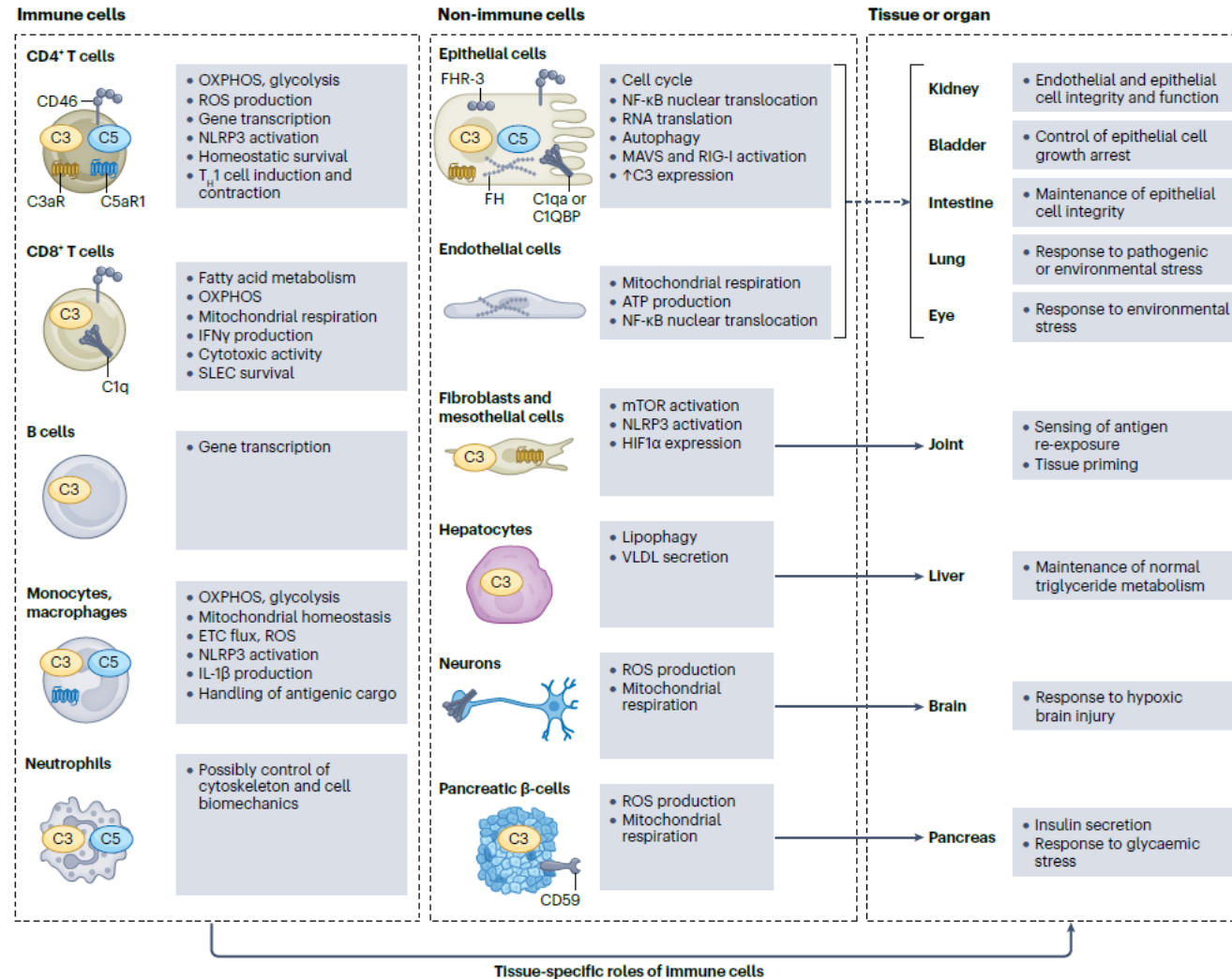
Ενδοκυττάριο Σύστημα Συμπληρώματος (Complosome)



Σύστημα Συμπληρώματος: Τροποποίηση της Βιολογίας και της Ομοιόστασης των Τ λεμφοκυττάρων



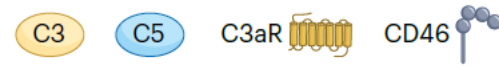
Ενδοκυττάριο Σύστημα Συμπληρώματος (Complosome) στα Κύτταρα και τους Ιστούς



Ενδοκυττάριο Σύστημα του Συμπληρώματος (composome): Ρόλος στην Ανθρώπινη Νόσο

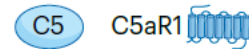
Infection and infection-associated pathology

- Respiratory tract infections (C3, C3aR, CD46)
- Immunodeficiency (C3)
- SARS-CoV-2-induced lung pathology (C3)



Cardiovascular disease

- Atherosclerosis (C5, C5aR1)
- Plaque destabilization (C5, C5aR1)



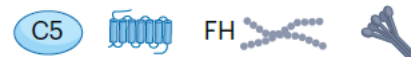
Cancer

- Colon cancer (C1q, C1qBP)
- Bladder cancer (CD46)



Kidney disease

- Renal endothelial dysfunction (FH)
- Injury-induced nephropathy (C5, C5aR1)
- Fibrosis (C5, C5aR1)
- Clear-cell renal cell carcinoma (FH, C1)



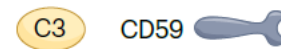
Autoimmunity – arthritic disease

- Rheumatoid arthritis (C3, CD46)
- SLE (C1q, C3, C5, CD46, FH)
- Scleroderma (C3, C5, C5aR1, CD46)



Diabetes and metabolic syndrome

- Diabetes (C3, CD59)
- Non-alcoholic fatty liver disease (C3)



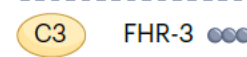
IBD

- Crohn's disease (C3, C3aR)

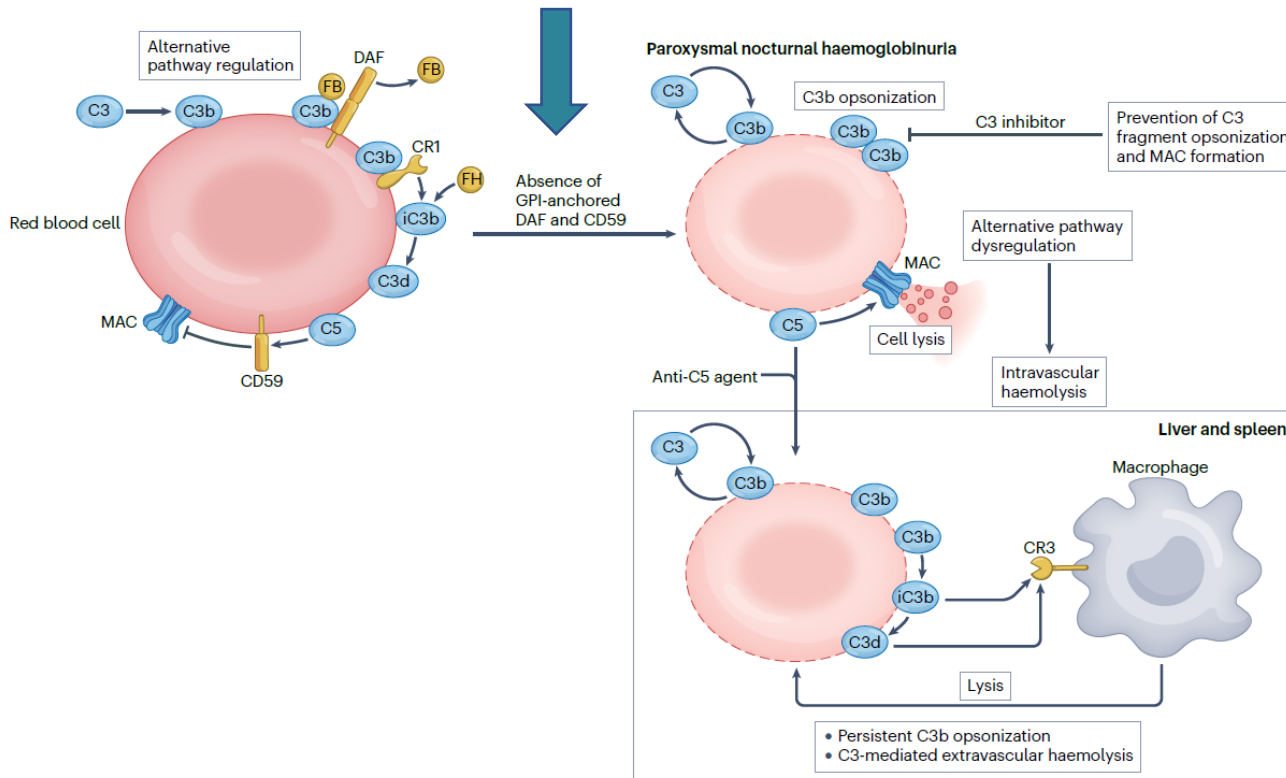


Potential composome-driven disease

- AMD (FHR-3)
- Ageing-related pathological conditions of tissue
- COPD, asthma
- C3 glomerulopathies, lupus nephritis
- Chronic neuroinflammation (for example, AD and MS)
- Diseases driven by defects in haematopoiesis



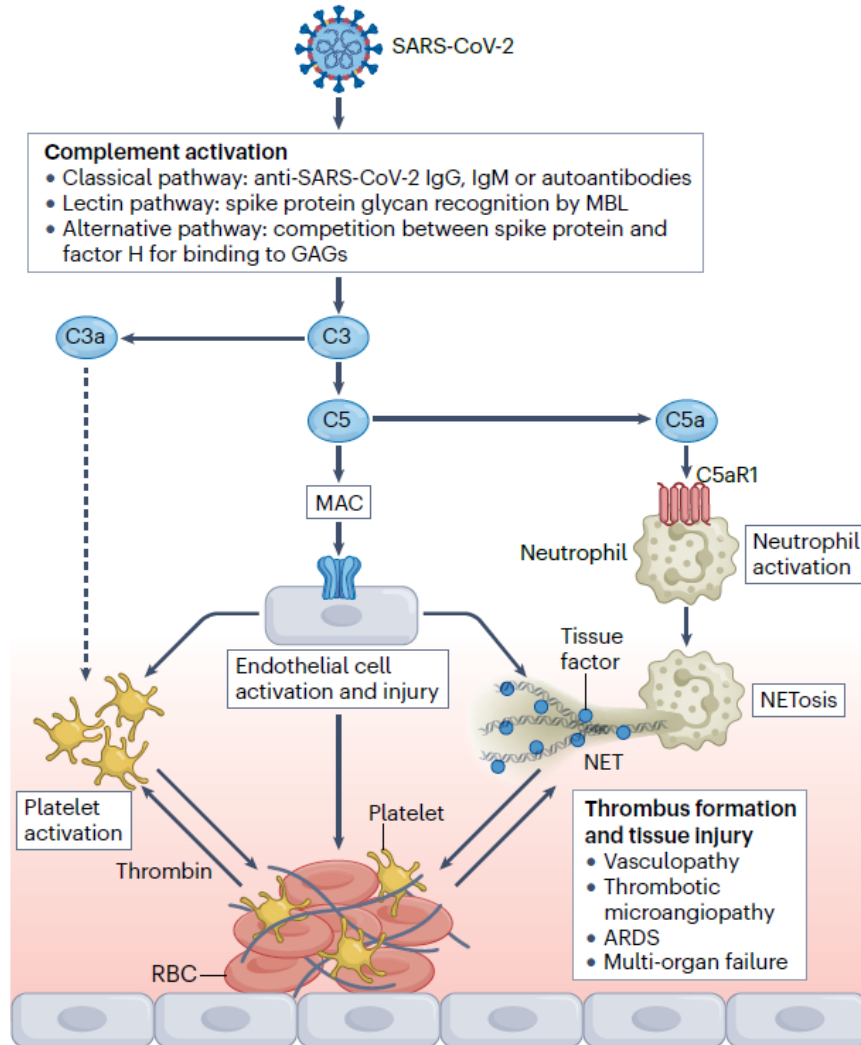
Η απορρύθμιση του συμπληρώματος πυροδοτεί την παθολογία στην PNH



- ❑ Νοσήματα ενδοαγγειακής αιμόλυσης:
 - Σύνδρομο υπεραιμόλυσης
 - aHUS

- ❑ Ενδοαγγειακή αιμόλυση → ↑ Αίμη → Ενεργοποίηση ΣΣ:
 - ↓ Factor I (↑ C3b)
 - ↓ έκφραση CD46, DAF
 - ↑ TLR4 εξαρτώμενη έκφραση της P-σελεκτίνης (↑ εναπόθεση C3b)

Complement dysregulation supports COVID-19-associated immunothrombosis



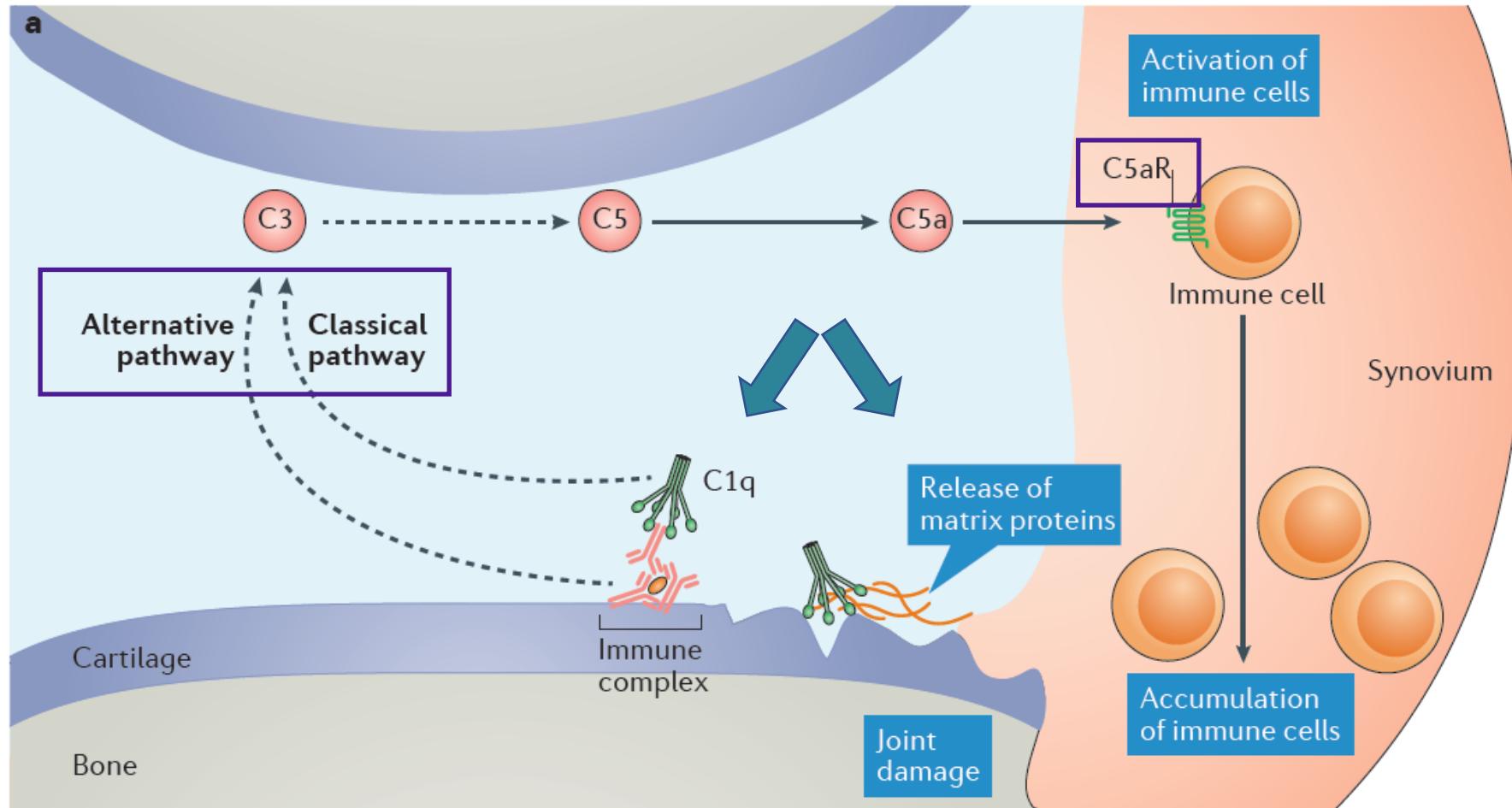
□ SARS-CoV-2:

- Ενεργοποίηση και των 3 οδών του ΣΣ

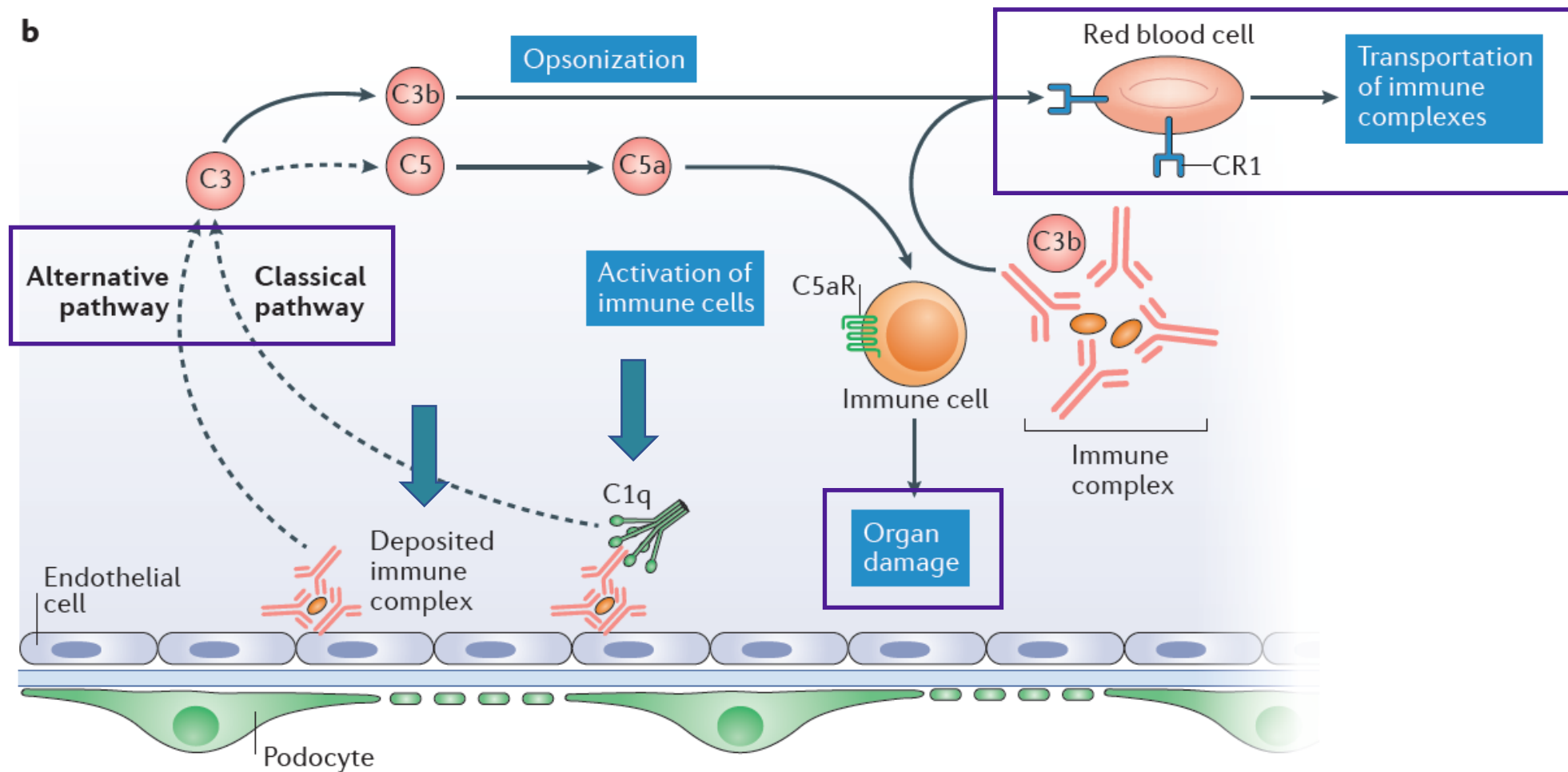
□ ↑ C3a, C5a, MAC

- ↑ ενεργοποίηση ενδοθηλίου
- Αγγειακή βλάβη
- Ανοσοθρόμβωση

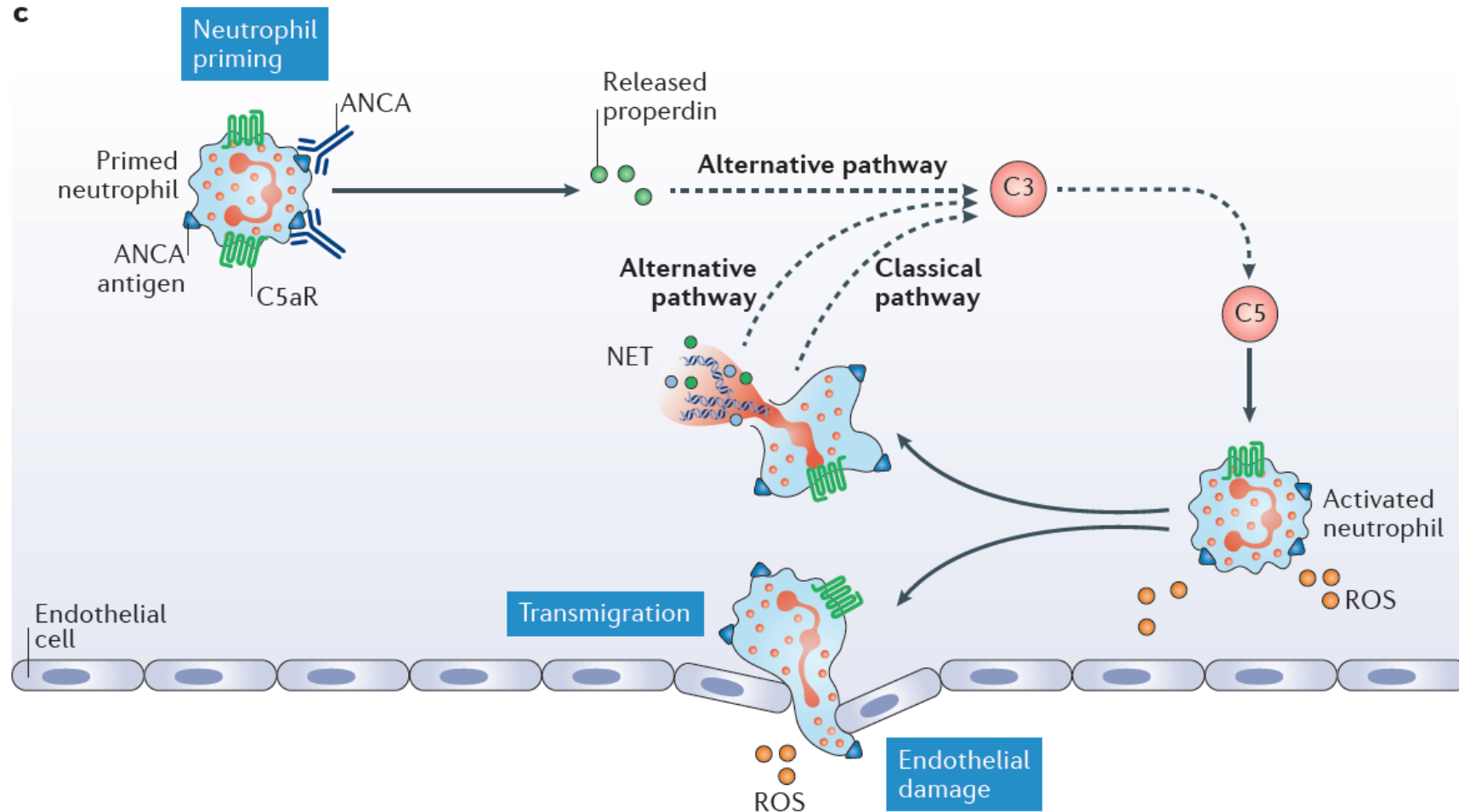
Ενεργοποίηση του συμπληρώματος: RA



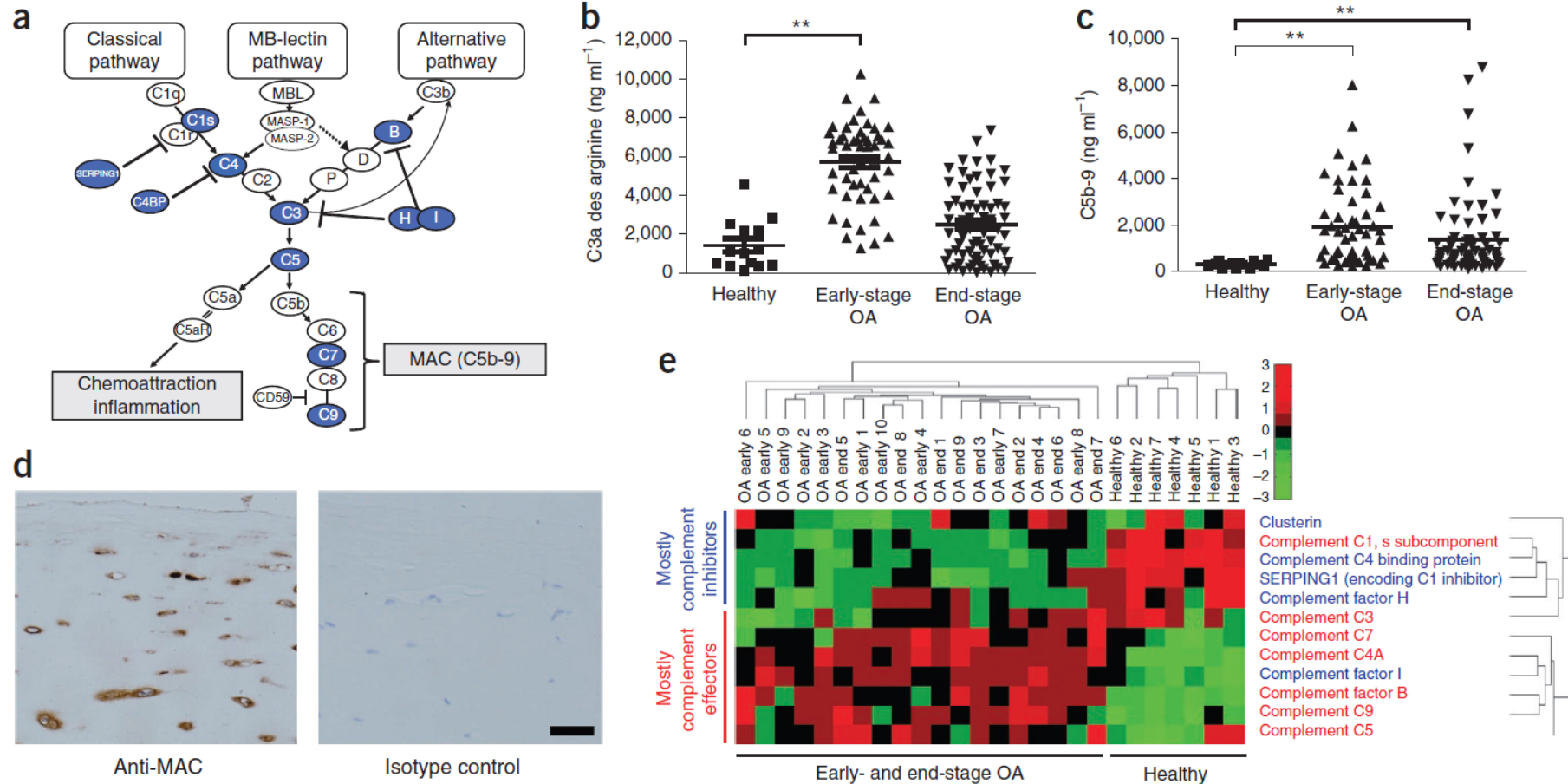
Ενεργοποίηση του συμπληρώματος: ΣΕΛ



Ενεργοποίηση του συμπληρώματος στις ANCA σχετιζόμενες αγγειίτιδες



Ενεργοποίηση του συμπληρώματος: OA

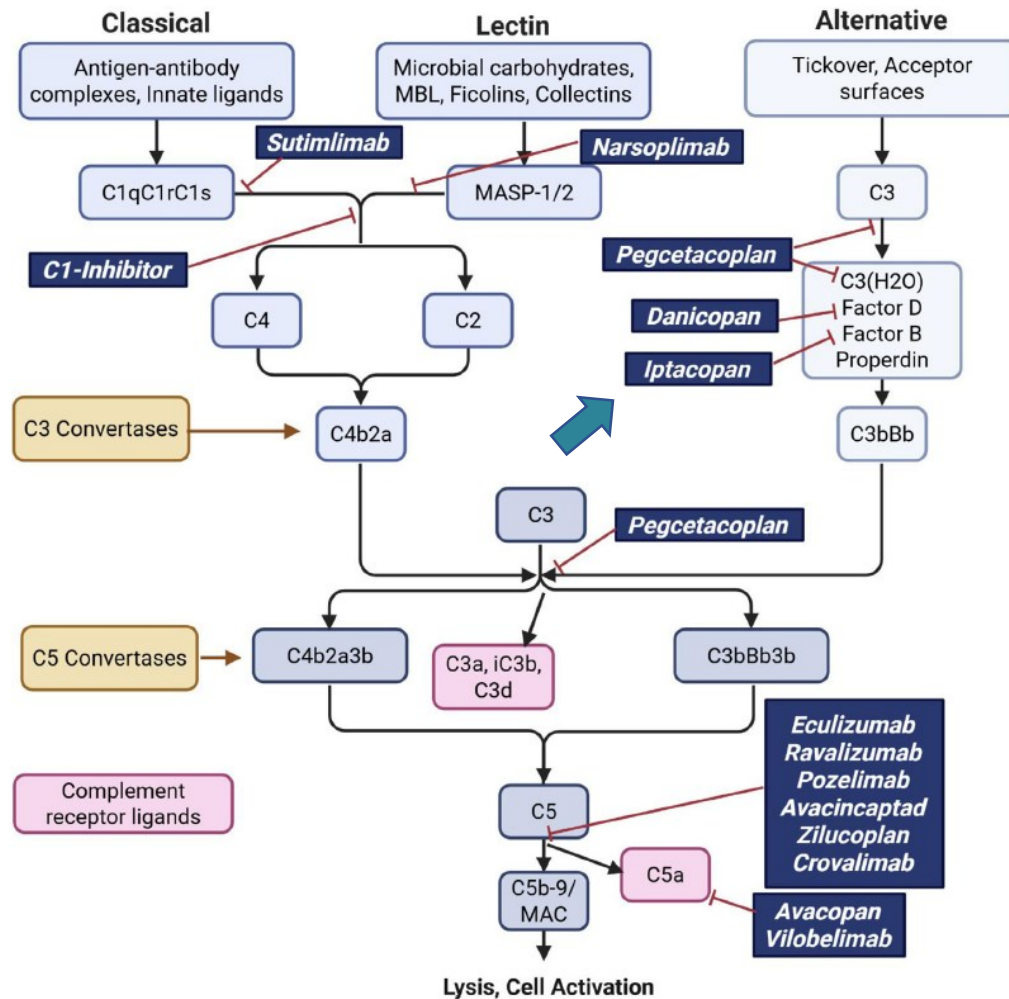


Νοσήματα που σχετίζονται με το σύστημα του συμπληρώματος και εγκεκριμένες θεραπείες

	Affected complement components	Complement-driven pathologies	Approved drug
Paroxysmal nocturnal haemoglobinuria	No regulators CD55 and CD59	Increased AP activity; MAC-mediated nocturnal lysis of RBCs (IVH); C3b, iC3b, or C3d deposition on RBCs (EVH in the liver)	Ecilizumab (anti-C5); ravulizumab (anti-C5); pegcetacoplan (C3 inhibitor)
Atypical haemolytic syndrome	LOF mutations in complement regulator genes (CD46, CFH, and CFI); GOF mutations (C3, CFB); autoantibodies against FH	Increased AP activity; MAC-mediated injury of kidney basal membrane; platelet activation (C5aR1); endothelial cell activation (C5aR1); TMA	Ecilizumab (anti-C5); ravulizumab (anti-C5)
Geographic atrophy secondary to age-related macular degeneration	Rare variants in complement genes (C3, C9, CFB, CFI, CFH, CD46, etc)	Increased AP activity; MAC-mediated death of retinal epithelial cells and photoreceptors; increased ocular recruitment of immune cells (C3aR and C5aRs)	Pegcetacoplan (C3 inhibitor)
ANCA-associated vasculitis	C1 activation by autoantibodies to proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA) expressed by neutrophils	CP activation with subsequent AP activation; neutrophil activation (ROS production) and NET formation (C5aR1); endothelial cell activation (C5aR1)	Avacopan (C5aR1 antagonist)
Neuromyelitis optica spectrum disorder	C1 activation by autoantibodies to aquaporin 4 expressed on cells of the CNS	CP activation with subsequent AP activation; MAC-mediated injury of astrocytes and subsequent neuronal damage	Ecilizumab (anti-C5)
gMG	C1 activation at neuromuscular interfaces by anti-AChR	CP activation with subsequent AP activation; MAC-mediated damage of cell membranes at the neuromuscular junctions	Ecilizumab (anti-C5) and ravulizumab (anti-C5)–both for anti-AChR+ gMG
Cold agglutinins disease	C1 activation by IgM antibodies recognising RBC antigens at temperatures $\leq 32^{\circ}\text{C}$	CP activation with subsequent AP activation; MAC-mediated lysis of RBCs (IVH); C3b, iC3b, or C3d deposition on RBCs (EVH in the liver)	Sutimlimab (anti-C1s)
COVID-19	Direct CP, LP, and AP activation by SARS-CoV-2 spike protein; SARS-CoV-2-induced type 2 pneumocyte local C3 production and activation	Endothelial and epithelial cell activation and injury (C5aR1, MAC); activation of the clotting cascade; immune cell recruitment and activation (C3aR and C5aRs)	Vilobelimab (anti-C5a); US Food and Drug Administration emergency use authorisation

AChR=anti-acetylcholine receptor antibody. ANCA= anti-neutrophil cytoplasmic antibody. anti-AChR=autoantibodies to the acetylcholine receptor. AP=alternative pathway. CP=classical pathway. EVH=extravascular haemolysis. FH=factor H. gMG=generalised myasthenia gravis. GOF=gain of function. IVH=intravascular haemolysis. LOF=loss of function. LP=lectin pathway. MAC=membrane attack complex. NET=neutrophil extracellular trap. RBC=red blood cell. ROS=reactive oxygen species. TMA=thrombotic microangiopathy.

Complement therapeutics and their targets in the complement pathway



Κατηγορίες:

- Θεραπείες ρυθμιστών του ΣΣ
- Αναστολείς των οδών ενεργοποίησης του ΣΣ
- Αναστολείς του C3 ή C5
- Αναστολείς των εκτελεστών ή υποδοχέων του ΣΣ

Ρευματολογικά νοσήματα και αναστολείς του συμπληρώματος ως θεραπευτική επιλογή

Disease	Approved therapeutic	Correlative human disease biomarker data	Primary clinical setting and/or positive case reports	Supportive murine model(s)
ANCA-associated vasculitis	Avacopan	Presence of histologic, urinary and circulating complement activation fragments [77,78]	Steroid sparing and clinical benefit in AAV with renal disease [66]	C5aR inhibition and alternative pathway deficiency impairs passive antibody transfer-induced renal disease [79,96]
Rheumatoid arthritis	No approval	Presence of histologic and circulating complement activation fragments [9]	Early RA [80]	Many pathway and receptor inhibitors (summarised in [9])
Lupus nephritis	No approval	Presence of histologic and circulating complement activation fragments [97]	Case reports with eculizumab in refractory systemic disease or lupus nephritis [84] TMA-like presentation	Inhibitors of alternative pathway, MAC and C5aR1 receptor [83,98]
Dermatomyositis	No approval	Presence of histologic complement activation fragments [81]	Not tested but likely best in clinically active disease [99]	NA
Antiphospholipid syndrome	No approval	Presence of circulating complement activation fragments [87]	Case reports with eculizumab in catastrophic antiphospholipid syndrome [88]	Classical and alternative pathway deficiency or receptor inhibitors [100,101]
IgA vasculitis	No approval	Presence of circulating complement activation fragments [81,82]	Not tested but likely in clinically active disease	NA
Scleroderma	No approval	Low factor levels potentially secondary to consumption	Chronic disease Case reports of renal crisis with eculizumab [85]	NA
SpA (PsA, axSpA)	No approval	Presence of circulating complement activation fragments [89,90]	Not tested but likely best in clinically active disease	Improvement with complement inhibition in model of ankylosing spondylitis [91]
Gout, crystalline arthritis	No approval	Ex vivo complement activation by crystals [92]	Not tested but likely best in clinically active disease	NA
Osteoarthritis	No approval	Presence of histologic and synovial fluid complement activation fragments [93]	Chronic disease [93]	Specific pathway or C5aR1 inhibitors [93]
Behcet's disease	No approval	Altered complement receptor levels [94]	Not tested but likely best in clinically active disease	NA
IgG4 disease	No approval	Hypocomplementaemia [95]	Not tested but likely best in clinically active disease	NA
Complement-activating conditions (CAC)	No approval	Variable complement biomarker associations [86]	Case reports of renal crisis with eculizumab [86]	NA

Σύστημα Συμπληρώματος: Ανεπάρκειες

Deficiency	Gene localisation	Results of complement analysis ²	Associated Symptoms/Disorders ³	OMIM database
Components				
C1q	1p36	CH50 C1 C1q	↓ SLE-like (> 90%, mostly clinically severe), infections	613652
C1r/s (mostly combined)	12p13	CH50 C1 C1r	↓ SLE-like, RA, infections	216950
C4 (C4A, C4B)	6p21	CH50 C4	↓ SLE-like, RA, infections, homozygous: severe symptoms; heterozygous: often clinically inapparent	614380 614379
C2	6p21	CH50 C2	↓ SLE-like, RA, infections (pneumonia), vasculitis, often clinically inapparent	217000
C3	19p13	CH50, AH50 C3	↓ Pyogenic infections	613779
C5	9q33-34	CH50, AH50 C5	↓ Meningitis (Neisseriae), SLE	609536
C6	5p13	CH50, AH50 C6	↓ Meningitis (Neisseriae), SLE	612446
C7	5p13	CH50, AH50 C7	↓ Meningitis (Neisseriae), SLE	610102
C8α-γ*/C8β	C8α/β: 1p32 C8γ: 9q34	CH50, AH50 C8	↓ Meningitis (Neisseriae), SLE	613790 613789
C9	5p14-p12	CH50, AH50 C9	↓ Neisserial infections (mostly asymptomatic)	613825
Factor B	6p21	AH50 FB	↓ Neisserial infections	615561
Factor D	19p13	AH50 FD	↓ Neisserial infections	134350
MBL	10q11	LP function MBL	↓ Bacterial infections (mostly asymptomatic)	614372
Ficolin 3 (H-Ficolin)	1p36	Ficolin	↓ Respiratory infections, necrotizing enterocolitis	613860
MASP-2	1p36	LP function MASP-2	↓ Respiratory infections	613791
MASP-3, CL-K1, CL-L1	1p36, 2q25, 3q27	MASP-3 CL-K1, CL-L1	↓ 3MC syndrome	257920, 265050, 248340
Regulators				
C1-Inhibitor	11q11-q13	C4 C1-INH	↓ Hereditary angioedema	106100
C4-binding Protein	1q32	C4BP	↓	120830 120831
Properdin	Xp11	(AH50) Properdin	↓ Meningitis (Neisseria),	312060
Factor H	1q32	<u>Homozygous:</u> CH50, AH50 C3 FH <u>Heterozygous:</u> CH50, AH50 n/C3 n/	↓ Infections, aHUS /C3G aHUS, C3G/MPGN	609814
FHR1 (FHR3)	1q32 (1q32)	CH50, AH50 n C3 n FH n	aHUS, RA, SLE often associated with factor H autoantibodies = > DEAP(deficiency of CFHR proteins and CFH autoantibody positive)-HUS	134371
Factor I	4q25	CH50, AH50 C3	↓ Infections (sepsis, meningitis, pneumonia) aHUS	610984
CD46/MCP	1q32	CH50, AH50 n CD46	↓ aHUS	120920
CD55/DAF	1q32	CH50, AH50 n CD55	↓ CHAPLE syndrome: Severe enteropathy Angiopathic thrombosis Complement hyperactivation Paroxysmale nocturnal hemoglobinuria (somatic mutation of PIG-A Gene**)	226300 125240
CD59	11p13 PIG-A: X	CH50, AH50 n CD59	↓ GBS-like symptoms, hemolysis Paroxysmale nocturnal hemoglobinuria (somatic mutation of PIGA Gene**)	612300 107271 300818
Receptors				
CR3 (CD18/CD11b)	CD18: 21q22	CD18/CD11b	↓ Leukocyte adhesion deficiency I (LAD I)	116920
CR4 (CD18/CD11c, LFA-1)	CD11b: 16p11 CD11c: 16p11	CD18/CD11c		600065

Σύγχρονες Μέθοδοι Αξιολόγησης της Δραστηριότητας των Οδών του Συμπληρώματος

❑ Περιορισμοί στην αξιολόγηση του ΣΣ στην κλινική πράξη:

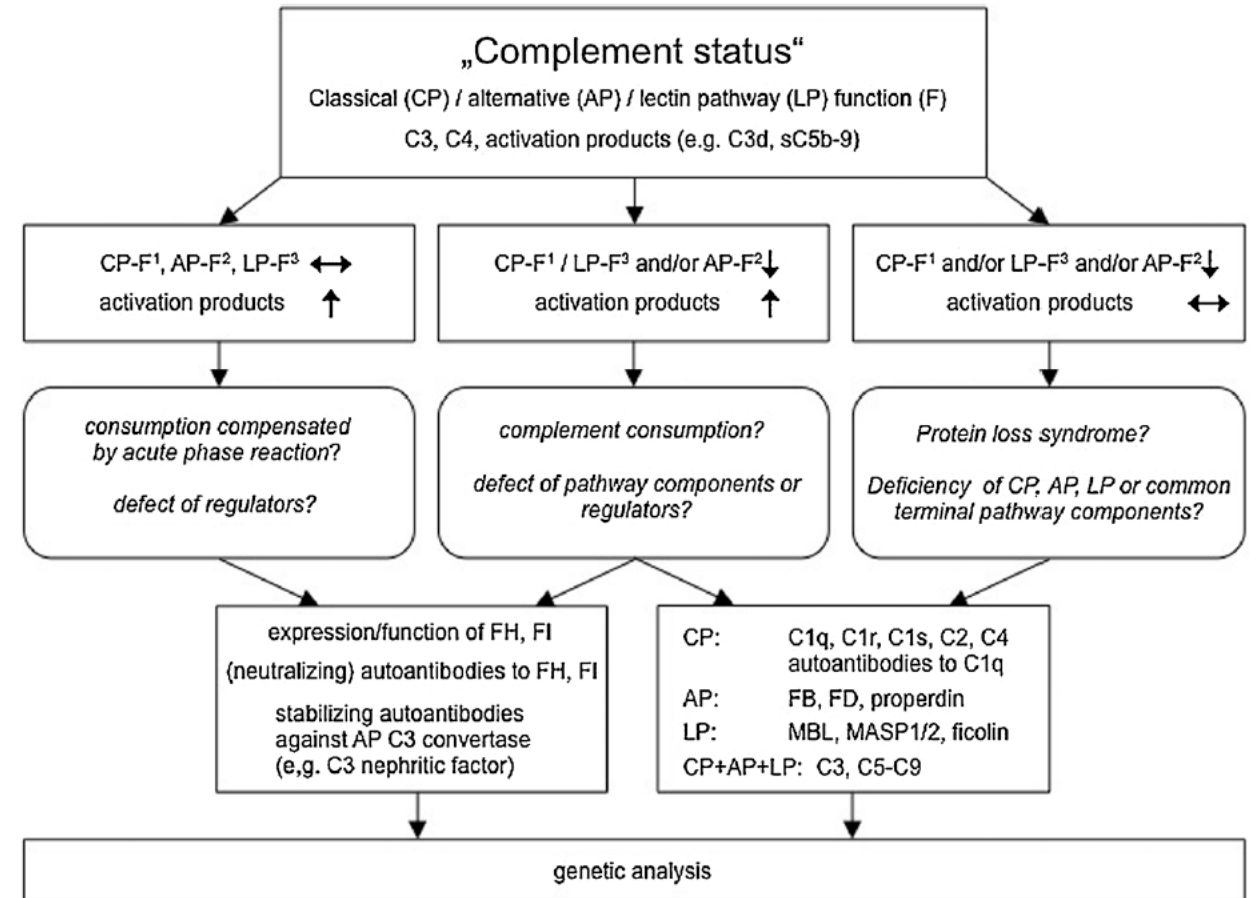
- Ανίχνευση ειδικών θραυσμάτων (π.χ. C3a, C5a, sC5-9)
- Αξιολόγηση κατανάλωσης – παραγωγής
- Εντοπισμός συγκεκριμένης έλλειψης
- Μέτρηση της αναστολής σε ασθενείς υπό θεραπεία (C3 αναστολείς)

Testing category	Factor(s) measured	Testing method(s) and strategies	Clinical setting example(s)	Example(s) of clinical utility
Pathway component level	C3 C4	ELISA	Complement deficiency SLE aHUS Other complementopathies	Diagnosis Disease flare Prognosis
Pathway function	CH50 AP50 sC5b-9 generation (all pathways)	Haemolysis and/or ELISA activation measure	Complement deficiency Therapeutic effect	Diagnosis Disease flare
Activation products	C1q C3a C5a iC3b, C3d C3c C4d	ELISA Immunohistochemistry	Lupus nephritis AAV Antibody-mediated transplant rejection Glomerulopathies	Disease flare Prognosis
Protein complexes	sC5b-9 C3 and C5 convertases	ELISA Immunohistochemistry	Lupus nephritis C3 glomerulopathies	Diagnosis Disease flare
Genetics	FH MCP FI Properdin C3 FB CFHR FH	DNA sequencing	aHUS C3 glomerulopathy Infection	Diagnosis
Autoantibodies	Anti-C1q Anti-FH C3 and C5 nephritic factors	ELISA Function	Lupus nephritis aHUS C3 glomerulopathy	Diagnosis Prognosis
Cell-fixed activation fragments	Cell-bound complement activation products	Flow cytometry	SLE	Diagnosis Prognosis

Εργαστηριακή ανάλυση των διαταραχών του συστήματος του συμπληρώματος

❑ Red Flags

- ❑ Μηνιγγιτιδοκοκκική μηνιγγίτιδα σε ηλικίες > 5 ετών
- ❑ Υποτροπιάζουσες συστηματικές λοιμώξεις από βακτήρια με κάψα
- ❑ Αυτοάνοσα νοσήματα (ΣΕΛ)
- ❑ Αγγειοίδημα απουσία κνίδωσης
- ❑ Φλεγμονώδη νοσήματα των νεφρών/οφθαλμών



¹ function of the classical pathway (CH50)

² function of the alternative pathway (AH50)

³ function of the lectin pathway

Take home messages

- ✓ Το σύστημα του συμπληρώματος είναι ευρέως κατανοημένο στον οργανισμό
- ✓ Η θέση της δραστηριότητας του συμπληρώματος φαίνεται να καθορίζει τις ποικίλες λειτουργίες του.
- ✓ Η αναστολή του συμπληρώματος αποτελεί μια υποσχόμενη θεραπευτική επιλογή στα ρευματικά νοσήματα





Σας Ευχαριστώ