Role of the Immune System in Diabetic Kidney Disease

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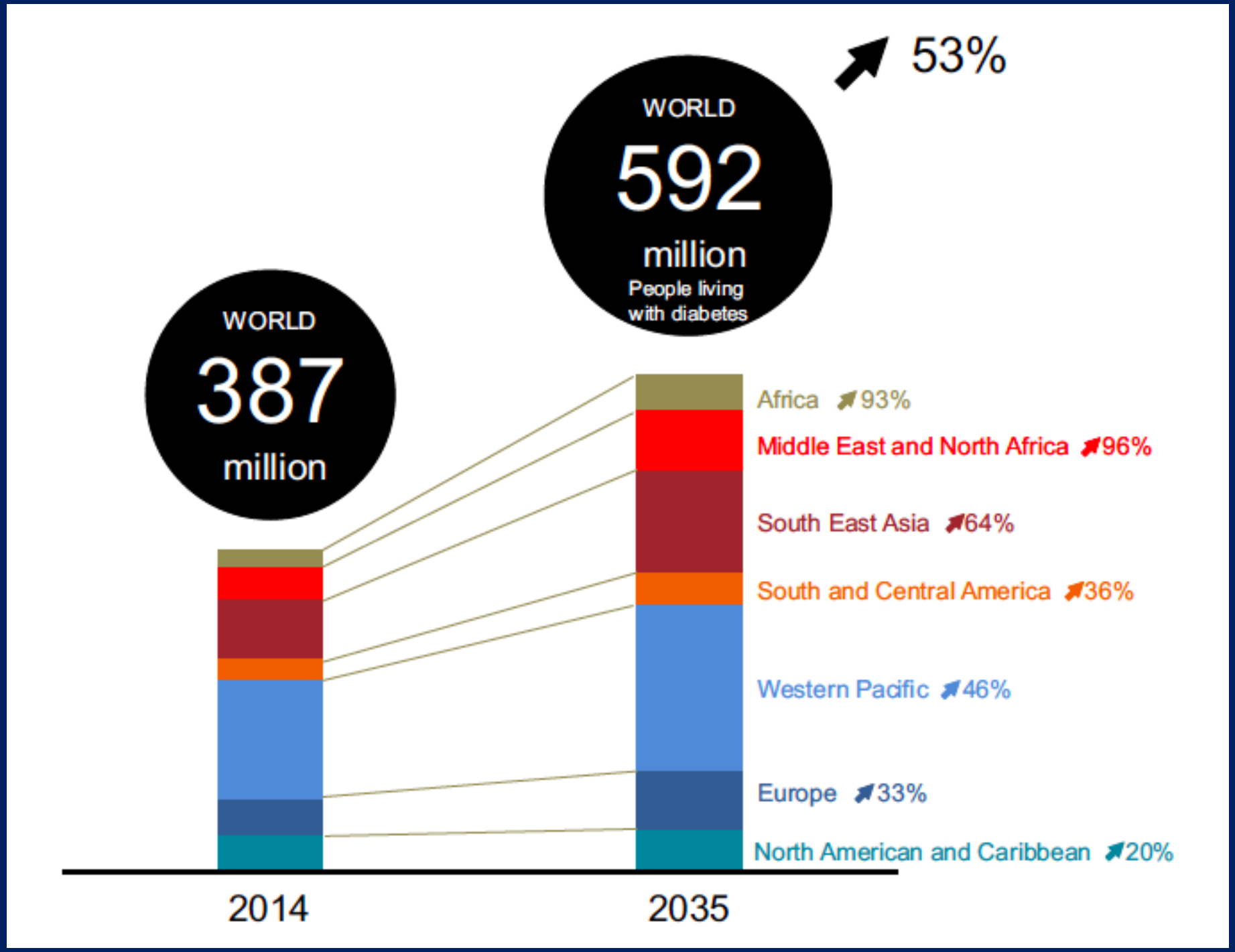
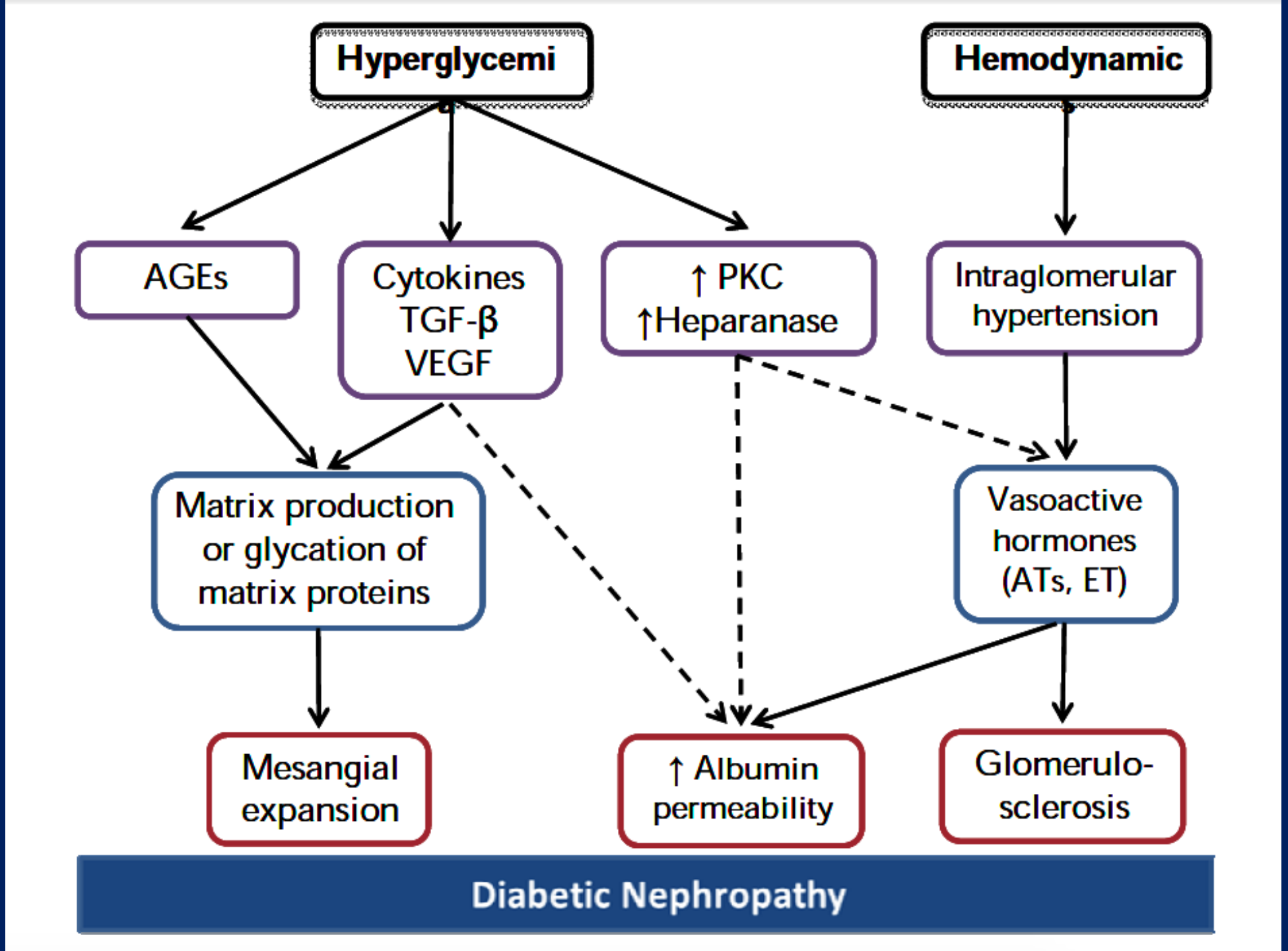
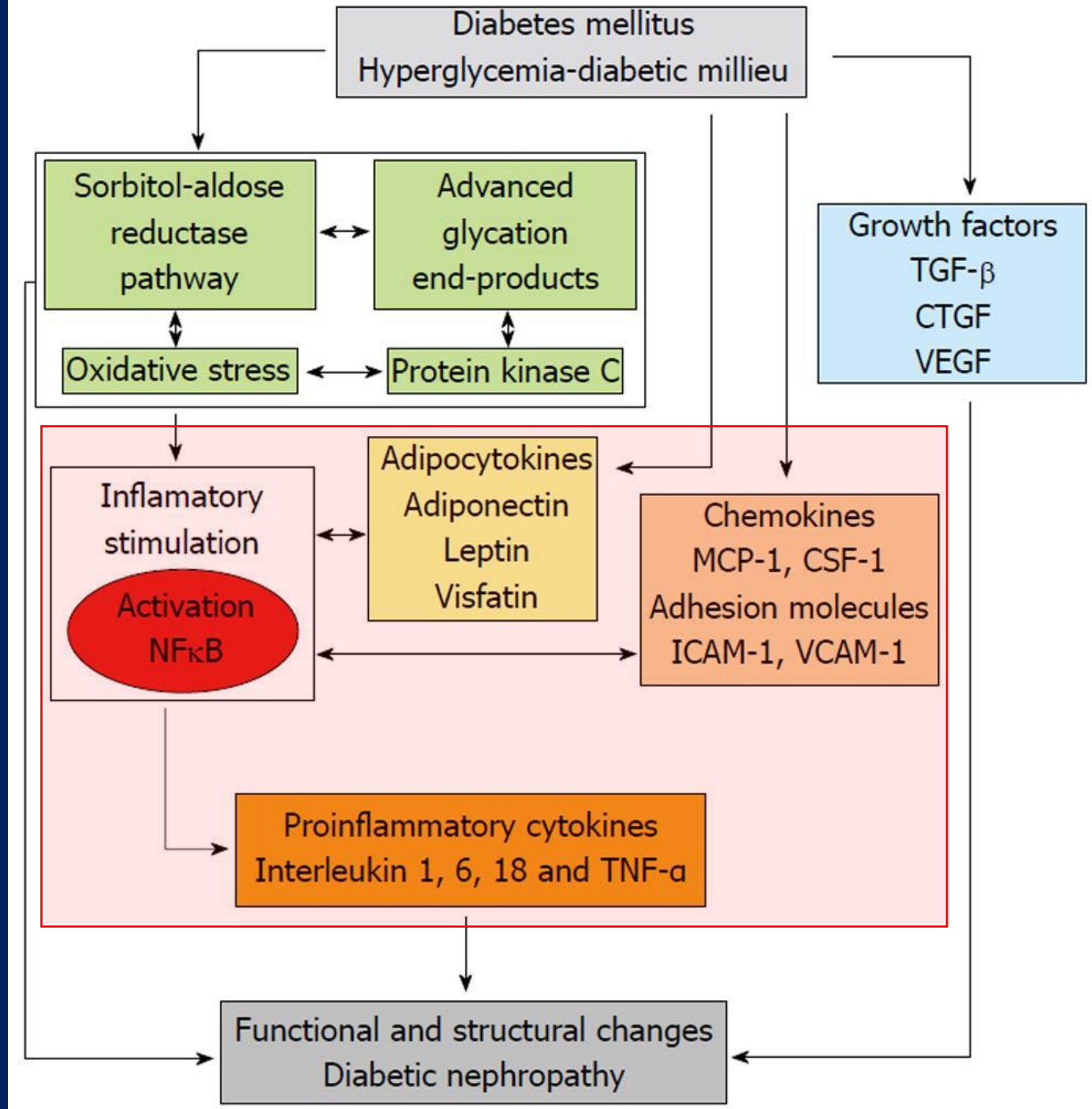

53%
WORLD
592
million
People living
with diabetes
WORLD
387
million
Africa 93%
Middle East and North Africa 96%
South East Asia 64%
South and Central America 36%
Western Pacific 46%
Europe 33%
North American and Caribbean 20%
2014
2035


Hyperglycemi
Hemodynamic
AGEs
Cytokines
TGF-β
VEGF
↑ PKC
↑Heparanase
Intraglomerular
hypertension
Matrix production
or glycation of
matrix proteins
Vasoactive
hormones
(ATs, ET)
Mesangial
expansion
↑ Albumin
permeability
Glomerulo-
sclerosis
Diabetic Nephropathy


Diabetes mellitus
Hyperglycemia-diabetic millieu
Sorbitol-aldose reductase pathway
Advanced glycation end-products
Oxidative stress
Protein kinase C
Growth factors
TGF-β
CTGF
VEGF
Inflamatory stimulation
Activation NFκB
Adipocytokines
Adiponectin
Leptin
Visfatin
Chemokines
MCP-1, CSF-1
Adhesion molecules
ICAM-1, VCAM-1
Proinflammatory cytokines
Interleukin 1, 6, 18 and TNF-α
Functional and structural changes
Diabetic nephropathy

Innate & Adaptive Immunity

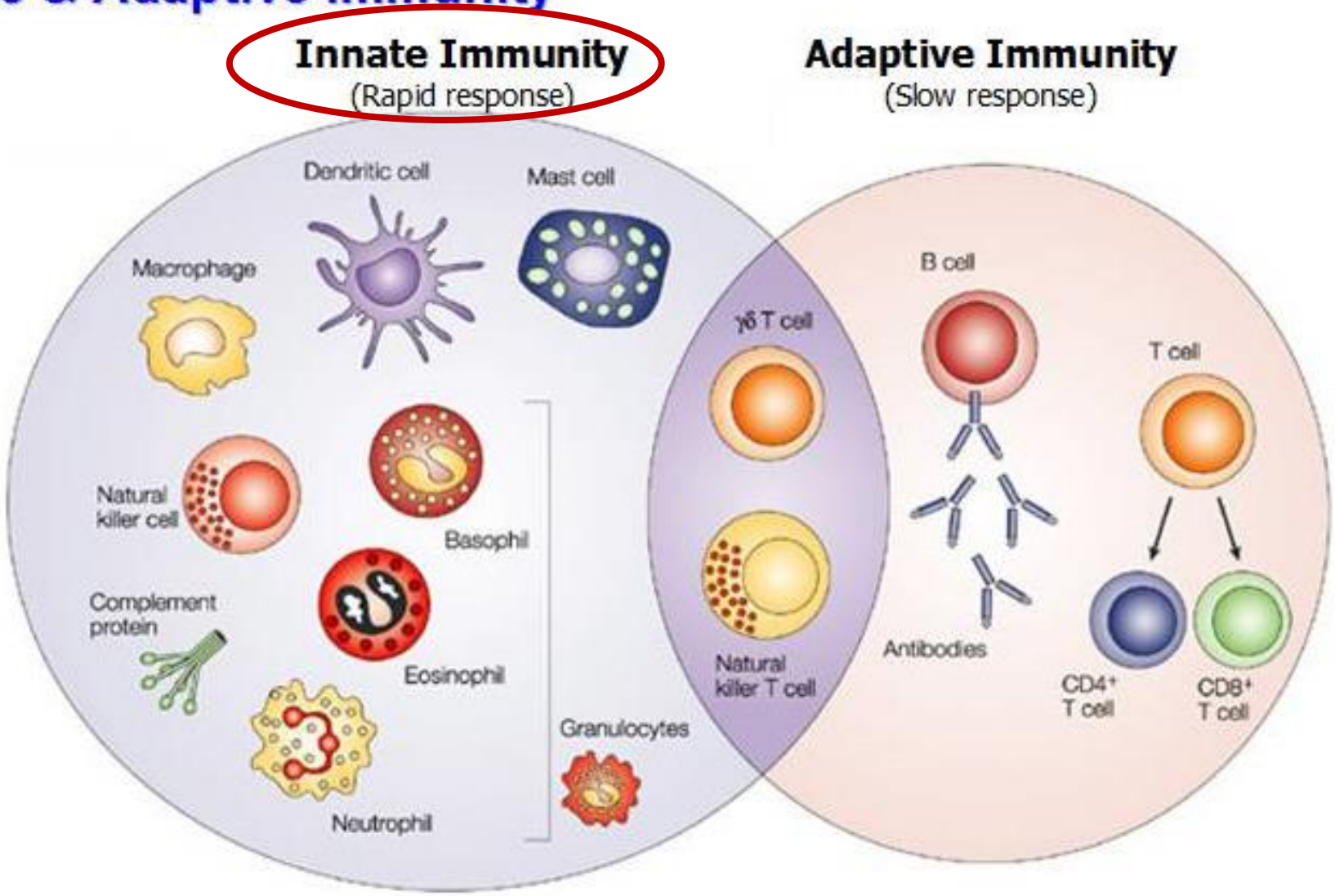
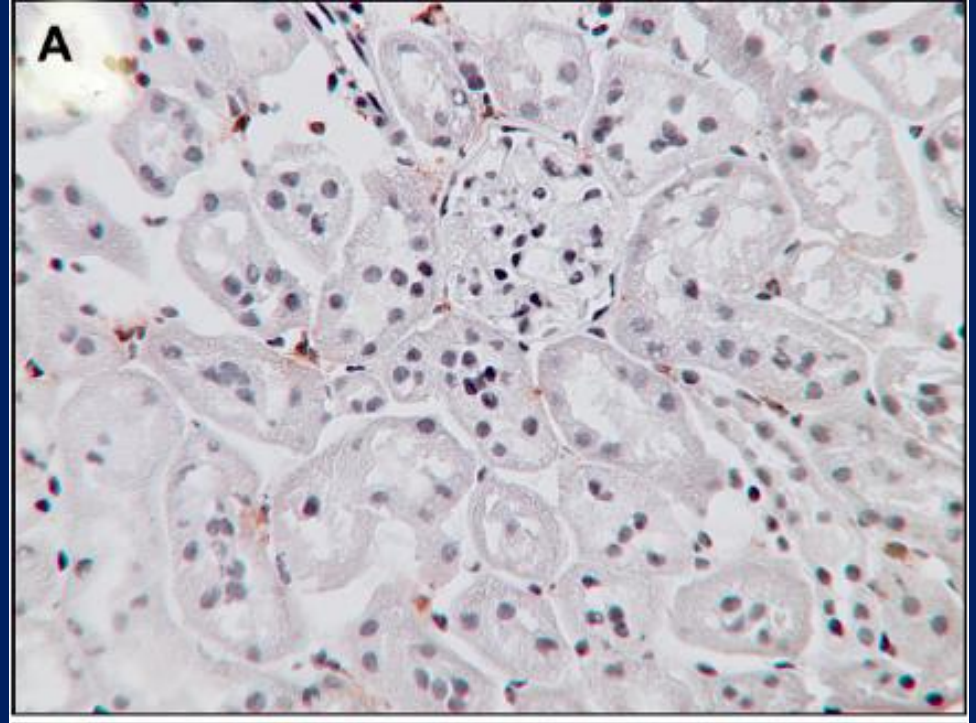


Table 1 | Classes of innate and adaptive pattern recognition molecules

Compartment	Innate recognition molecules	Adaptive recognition molecules
Secreted to extracellular fluids	Pentraxins (CRP, SAP, pentraxin-3) Complement factors Mannose-binding lectin	IgA, IgM, IgG, IgE
Cell surface	Mannose receptor Scavenger receptors Complement receptors Fc receptors Toll-like receptors Dectins	T-cell receptors B-cell receptors (Ig) Antigen-presenting molecules
Intracellular endosomes	Toll-like receptors	MHC I, MHC II
Intracellular cytosol	RIG-like helicases NOD-like receptors Inflammasome-activating molecules	

Abbreviations: CRP, C-reactive protein; Ig, immunoglobulin; MHC, major histocompatibility complex; NOD, nucleotide-binding oligomerization domain; RIG, retinoic-acid-inducible protein; SAP, serum amyloid P.

Role of Macrophages in Diabetic Nephropathy



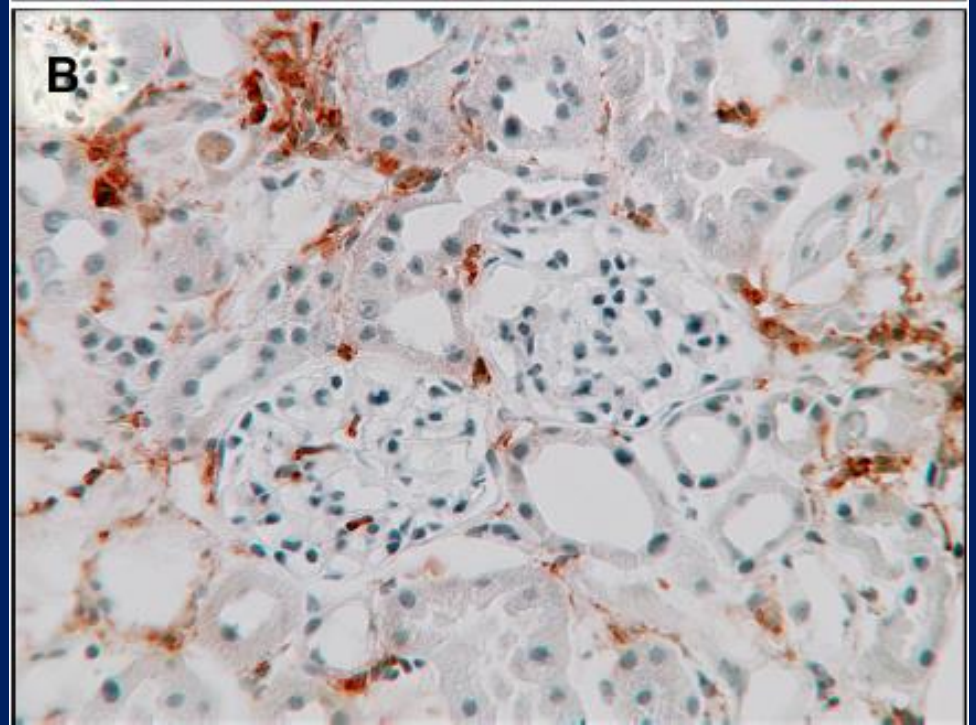


Table 3. Correlation between clinical parameters and interstitial CD68$^+$ macrophages

	ρ^a	P-value
GFR stage	0.302	0.009[b]
Serum creatinine (μmol/L)	0.317	0.006[b]
Presence of albuminuria	0.292	0.03[b]
Microalbuminuria and macroalbuminuria	0.293	0.03[b]
HbA1c	−0.266	0.14
Diabetes duration	−0.051	0.72
RAAS blockers	0.255	0.046[b]
Oral diabetic medication	−0.129	0.32

GFR stage, estimated glomerular filtration rate based on the KDIGO CKD guidelines; HbA1c, glycated haemoglobin; RAAS blockers, renin–angiotensin–aldosterone system blockers; NS, not significant, might be due to insufficient data.

[a]Spearman's correlation coefficient.

[b]Statistically significant ($P \leq 0.05$).

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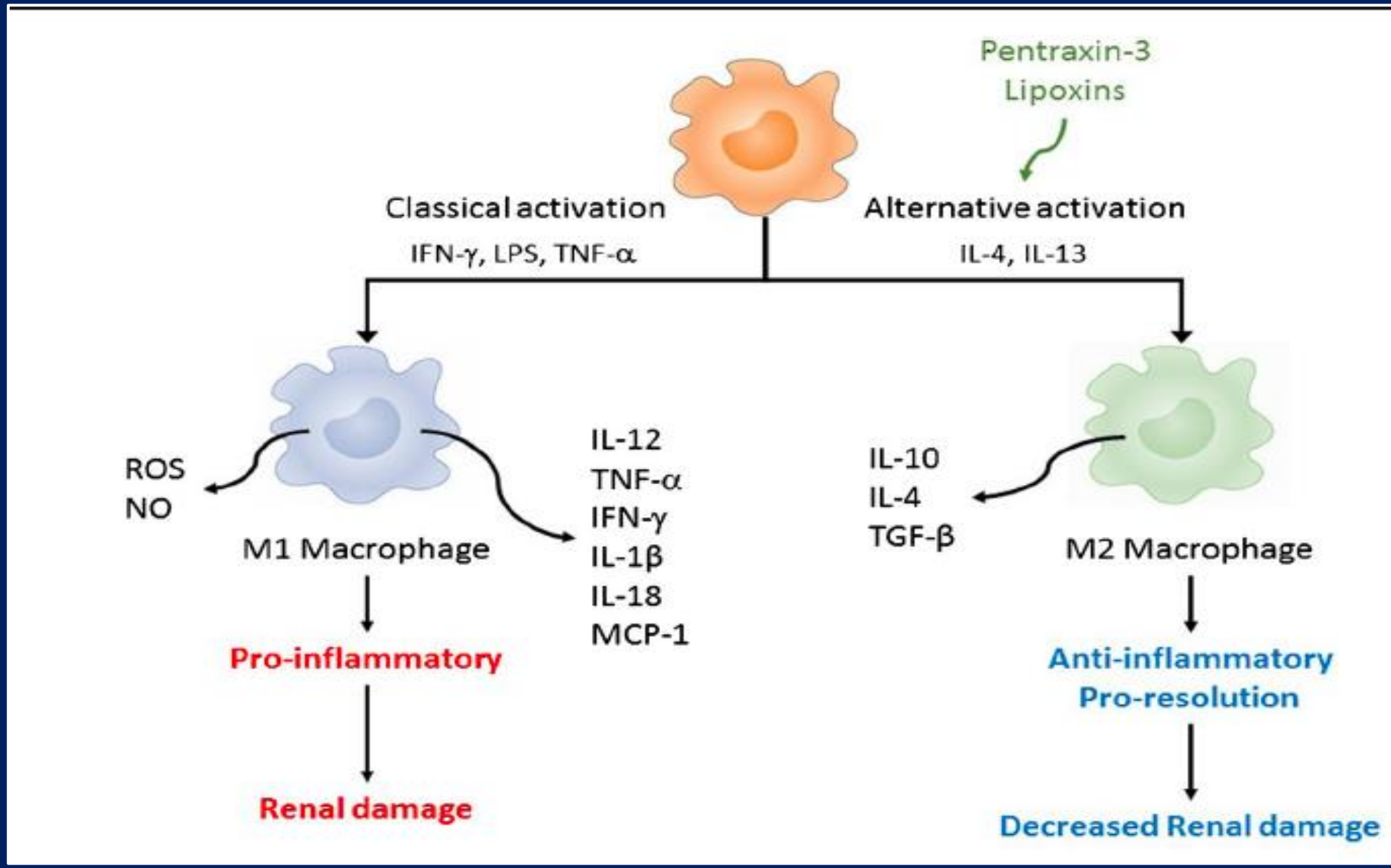
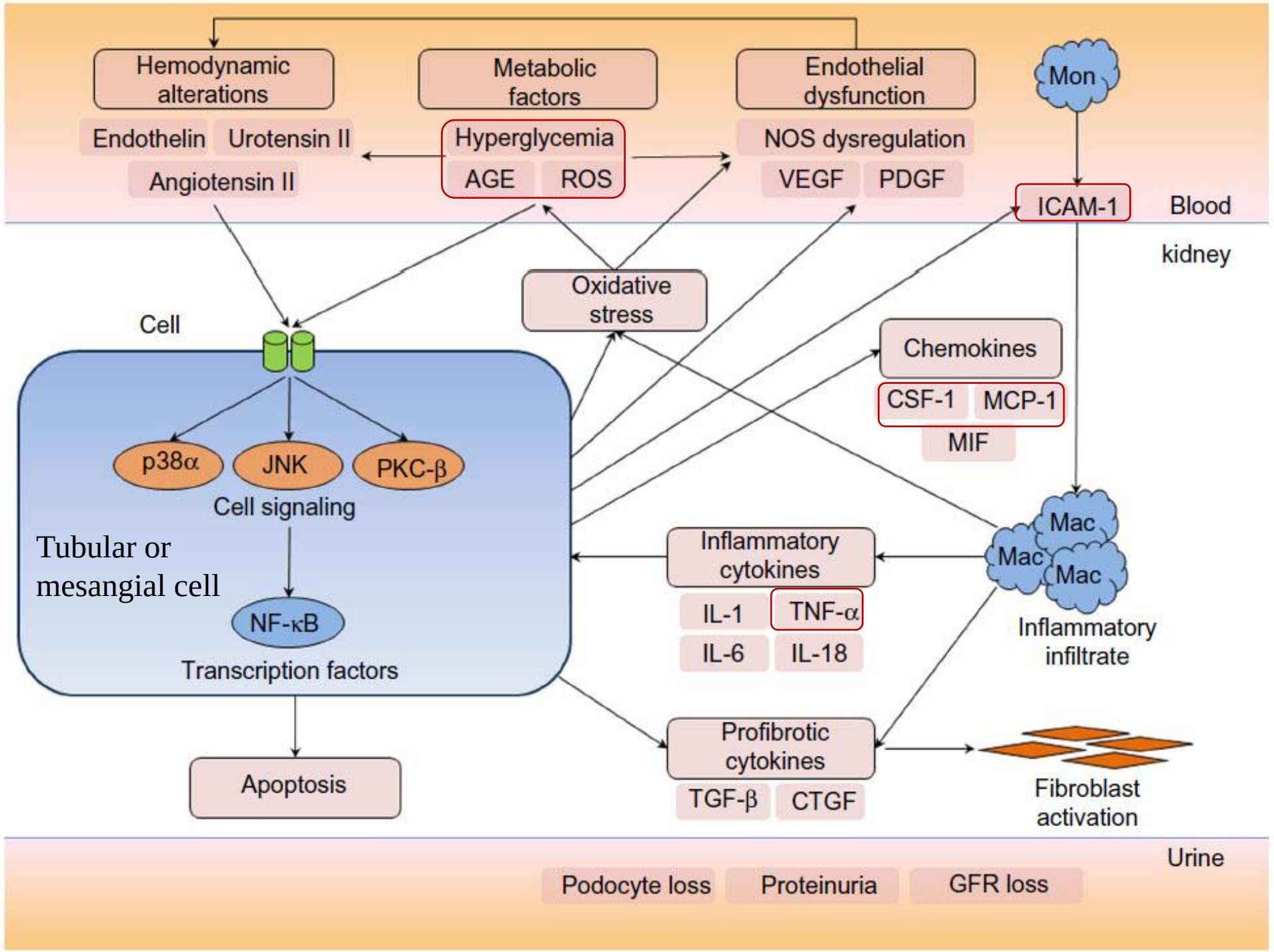
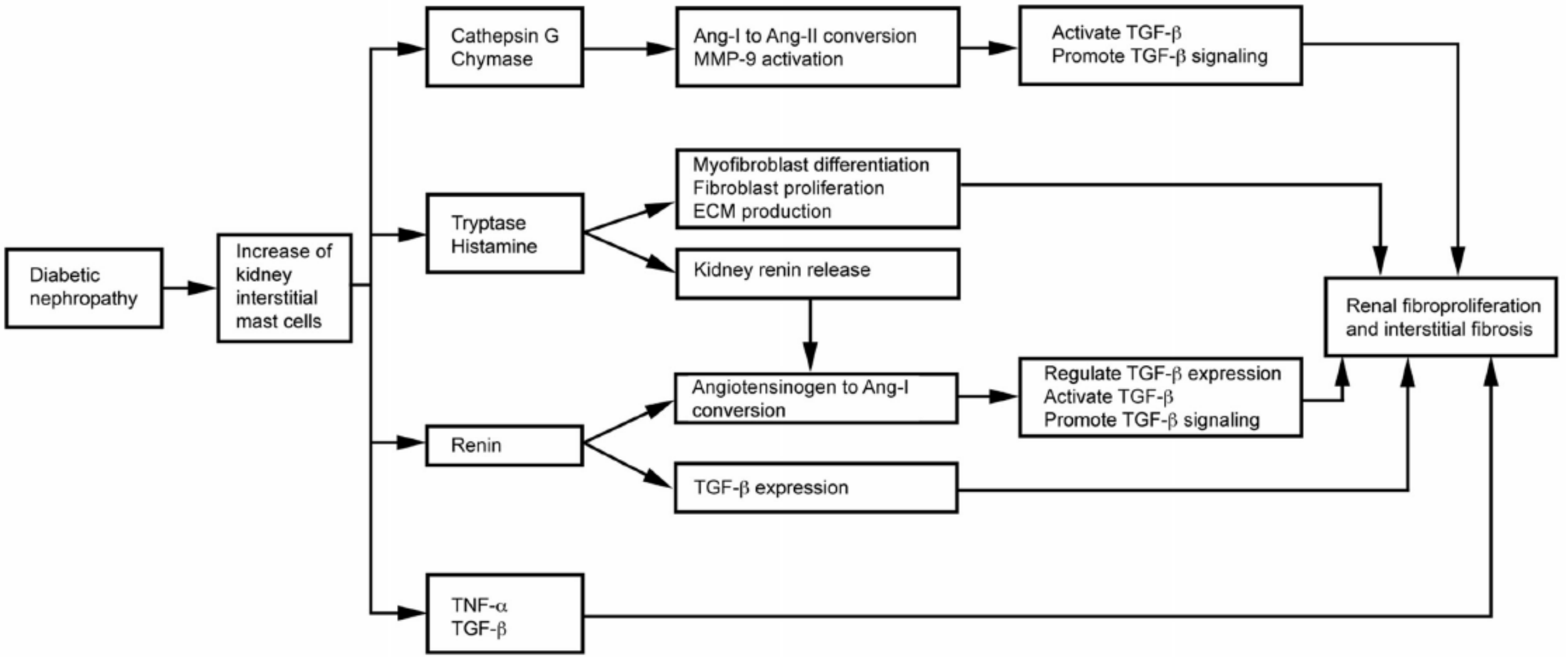

Pentraxin-3
Lipoxins
Classical activation
IFN-γ, LPS, TNF-α
Alternative activation
IL-4, IL-13
ROS
NO
M1 Macrophage
IL-12
TNF-α
IFN-γ
IL-1β
IL-18
MCP-1
IL-10
IL-4
TGF-β
M2 Macrophage
Pro-inflammatory
Renal damage
Anti-inflammatory
Pro-resolution
Decreased Renal damage


Hemodynamic alterations
Metabolic factors
Endothelial dysfunction
Mon
Endothelin
Urotensin II
Angiotensin II
Hyperglycemia
AGE
ROS
NOS dysregulation
VEGF
PDGF
ICAM-1
Blood
kidney
Oxidative stress
Cell
Chemokines
CSF-1
MCP-1
MIF
p38α
JNK
PKC-β
Cell signaling
Tubular or mesangial cell
NF-κB
Transcription factors
Inflammatory cytokines
IL-1
TNF-α
IL-6
IL-18
Mac
Mac
Mac
Inflammatory infiltrate
Apoptosis
Profibrotic cytokines
TGF-β
CTGF
Fibroblast activation
Urine
Podocyte loss
Proteinuria
GFR loss

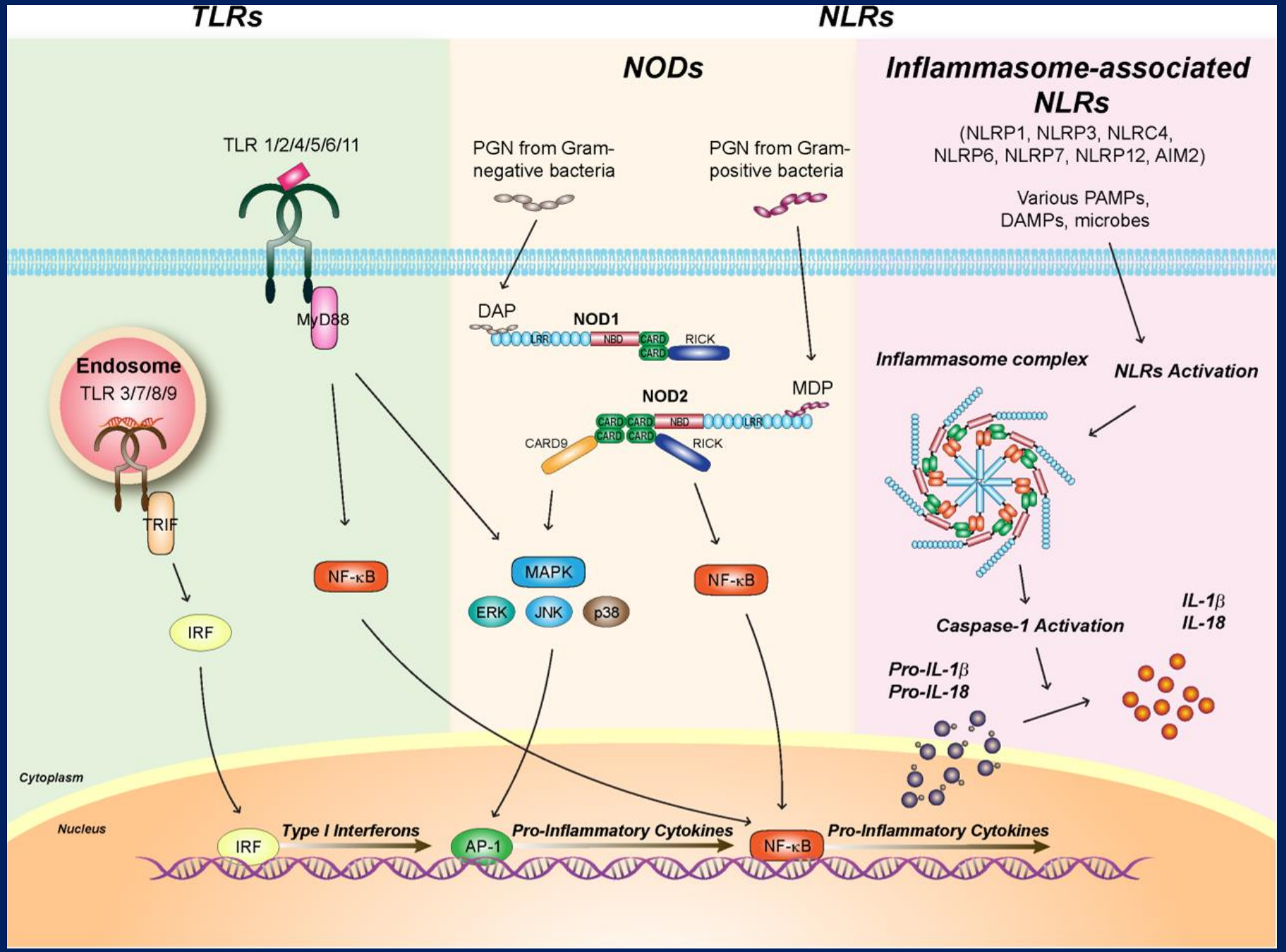
Evidence of Involvement in DKD	Experiment Type
MNP system cells	
MNPs accumulate in the diabetic kidney and their accumulation is associated with renal injury	
$CD11^+$ dendritic cells infiltrate glomeruli in NOD mice, proportional to albuminuria (210)	Animal models
Macrophages accumulate in kidneys in *db/db* mice (38, 39, 41) and early in diabetes in streptozotocin-treated animals (38, 170)	
Interstitial macrophage infiltration correlates with proteinuria (38, 39, 41, 170) as well as glomerular and tubular damage, renal fibrosis (38, 39, 41), MCP-1, M-CSF/CSF-1, and MIF (38)	
Macrophages are prominent in glomeruli and the interstitium of people with DKD (149). Their accumulation in glomeruli is proportional to glomerulosclerosis (64) and their interstitial infiltration predicts loss of GFR (149)	Human studies
Reducing macrophage infiltration of kidneys attenuates DKD	
Reduced renal macrophage infiltration in diabetic mice deficient in ICAM-1 (41), MCP1 (39), or its receptor CCR2 (21) attenuated renal fibrosis and albuminuria	Animal models
Macrophages produce cytokines and proteins that can cause tissue injury	
Macrophages treated with serum from diabetic rats promoted fibroblast proliferation via IL-1 and PDGF (38)	Animal models
IL-1β from macrophages stimulates human renal fibroblast proliferation and collagen I, fibronectin, TGF-β, and nitric oxide production. This was attenuated by anti-TGF-β (205). Macrophage IL-1 also stimulates mesangial cell proliferation (178)	
Mouse macrophages under high glucose produce IL-12, IL-1β, IL-18, TNF-α, TGF-β (213)	
Macrophages under high glucose or AGE produced more active TGFβ (36)	
AGE-modified proteins and oxidized glycated LDL induce release of IL-1β, TNF-α (211) and reactive oxygen species (95) from macrophages	

Role of Mast Cells in Diabetic Nephropathy

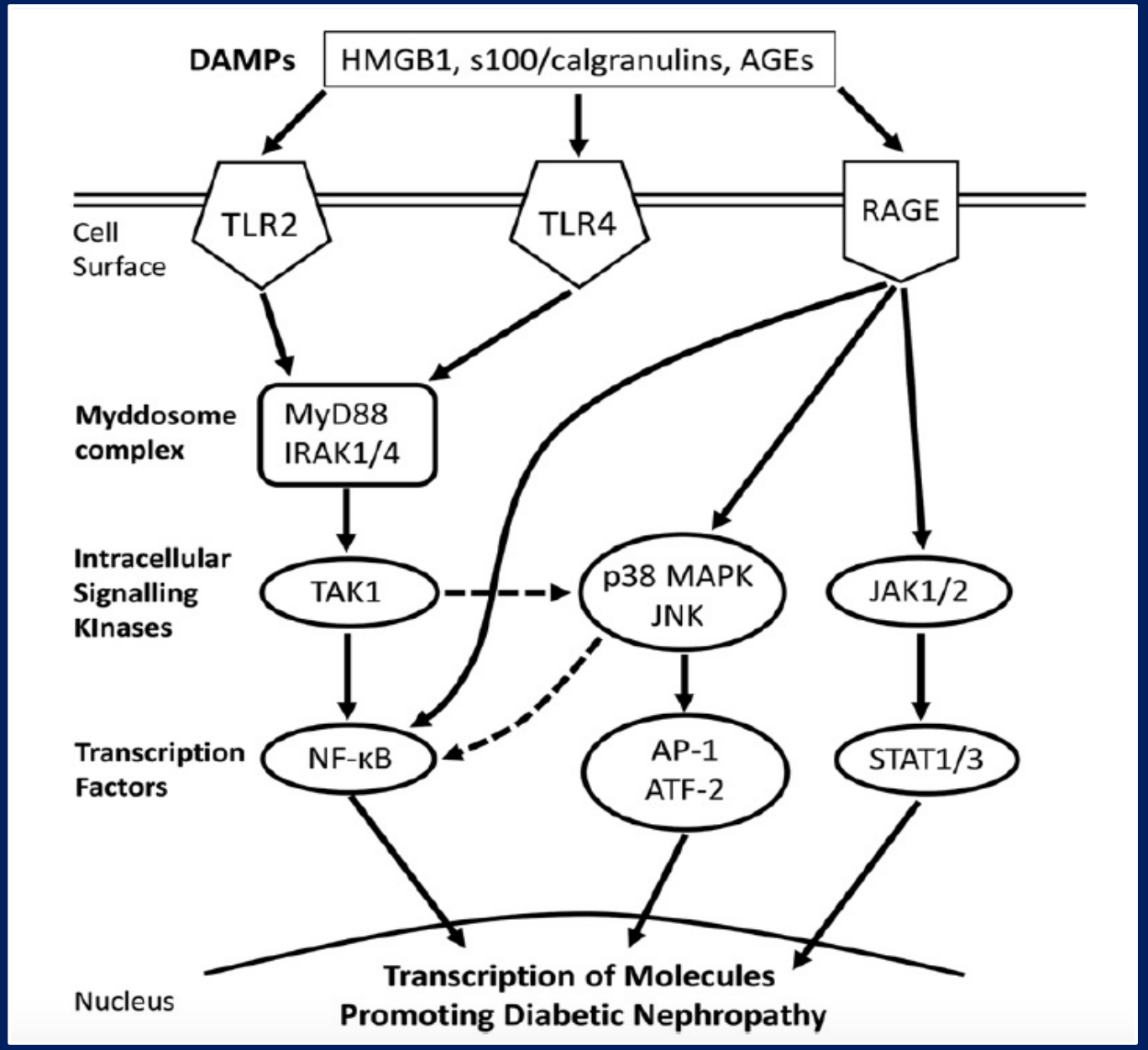

Diabetic nephropathy
Increase of kidney interstitial mast cells
Cathepsin G
Chymase
Ang-I to Ang-II conversion
MMP-9 activation
Activate TGF-β
Promote TGF-β signaling
Tryptase
Histamine
Myofibroblast differentiation
Fibroblast proliferation
ECM production
Kidney renin release
Renin
Angiotensinogen to Ang-I conversion
Regulate TGF-β expression
Activate TGF-β
Promote TGF-β signaling
TGF-β expression
TNF-α
TGF-β
Renal fibroproliferation and interstitial fibrosis

Mast cells	
Mast cells accumulate in DKD	
Mast cell density increased in interstitium of DKD (72, 82, 155, 166), correlated with interstitial volume and injury and serum creatinine (82, 155), but not urine protein (82)	Human studies
Mast cell number and degranulation increased with degree of DKD, correlating with interstitial injury. Most renal mast cells stained for chymase, renin, TGF-β, TNF-α (223)	
Chymase expression and activity increased in streptozotocin-treated rats. High-glucose treatment of mesangial cells led to higher chymase activity, expression of fibrosis markers, and TGF-β, and this was inhibited by chymostatin and Losartan (44)	
Preventing mast cell degranulation (mast cell stabilization) ameliorates DKD	
In streptozotocin-treated rats, agents that stabilized mast cells improved renal collagen content, urine protein, and glomerular filtration rate (88)	Animal models
Tranilast, an agent that stabilizes mast cells, reduced slope of 1/serum creatinine, but no change in proteinuria, in s small trial (9 patients with advanced DKD) (177)	Human studies
Mast cells degranulation releases mediators that can cause tissue injury	
Mast cells produce renin (37)	Animal models
Chymase inhibition reduces renal angiotensin II, fibronectin, TGF-β, mesangial expansion, and albuminuria more effectively than an ACE inhibitor (114)	
Human mast cell tryptase stimulated proliferation and type 1 collagen synthesis in human lung (31) and dermal fibroblasts (73)	Human models

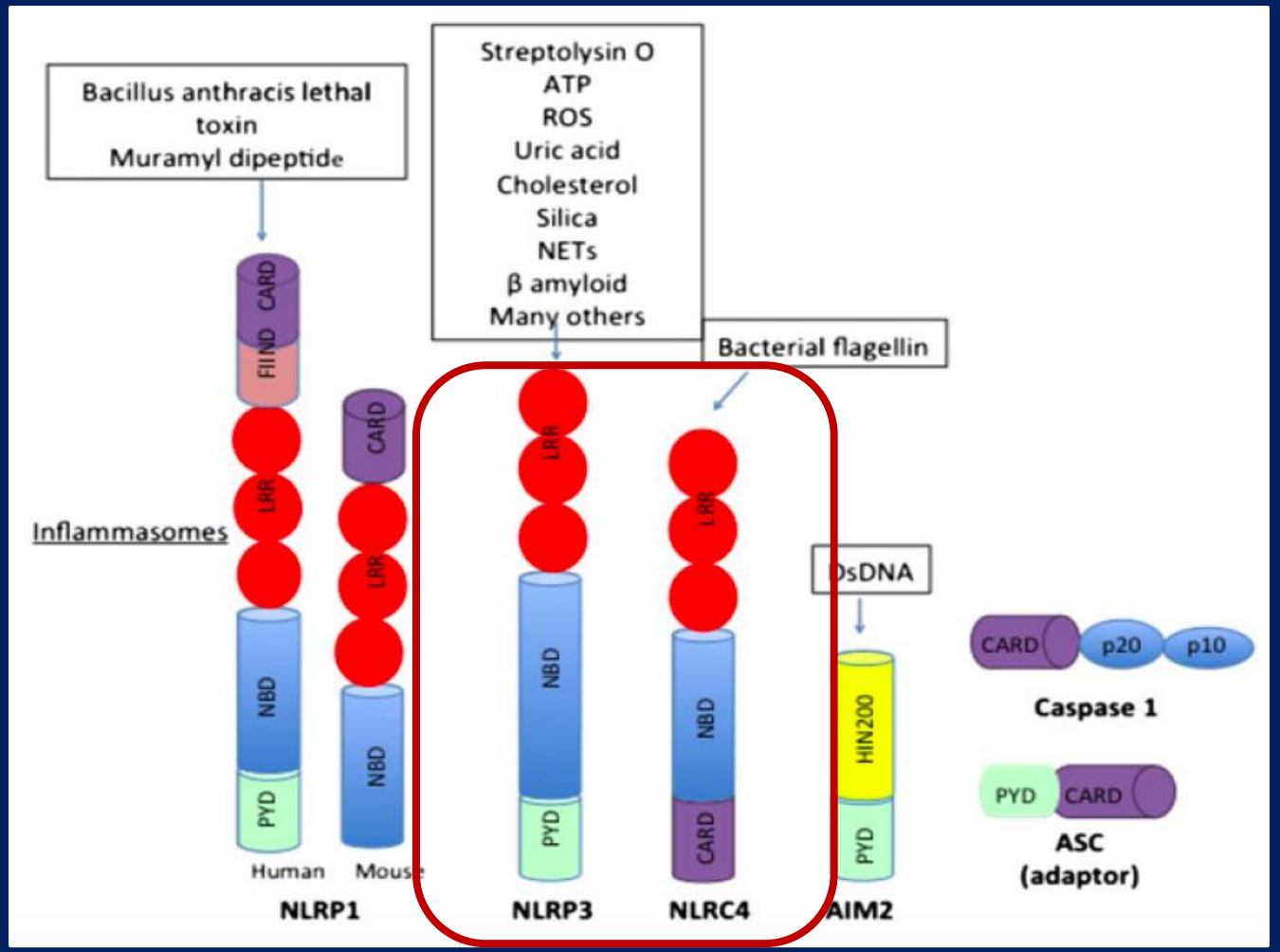
Pattern Recognition Receptors in Diabetic Nephropathy

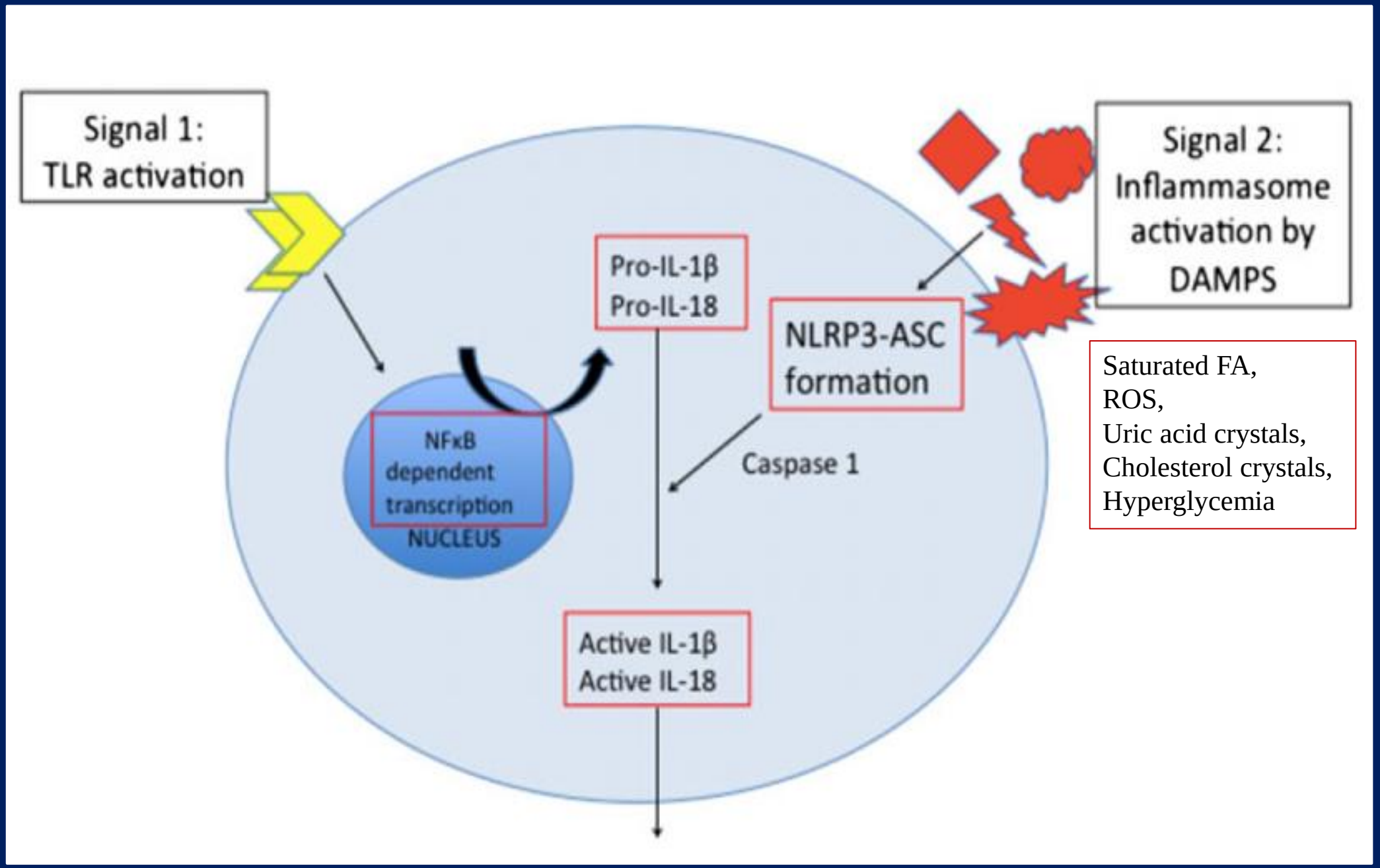

TLRs
NLRs
NODs
Inflammasome-associated NLRs
(NLRP1, NLRP3, NLRC4, NLRP6, NLRP7, NLRP12, AIM2)
TLR 1/2/4/5/6/11
PGN from Gram-negative bacteria
PGN from Gram-positive bacteria
Various PAMPs, DAMPs, microbes
MyD88
DAP
NOD1
LRR
NBD
CARD
CARD
RICK
Endosome
TLR 3/7/8/9
TRIF
NOD2
MDP
CARD9
Inflammasome complex
NLRs Activation
NF-κB
MAPK
ERK
JNK
p38
NF-κB
IRF
Caspase-1 Activation
IL-1β
IL-18
Pro-IL-1β
Pro-IL-18
Cytoplasm
Nucleus
IRF
Type I Interferons
AP-1
Pro-Inflammatory Cytokines
NF-κB
Pro-Inflammatory Cytokines

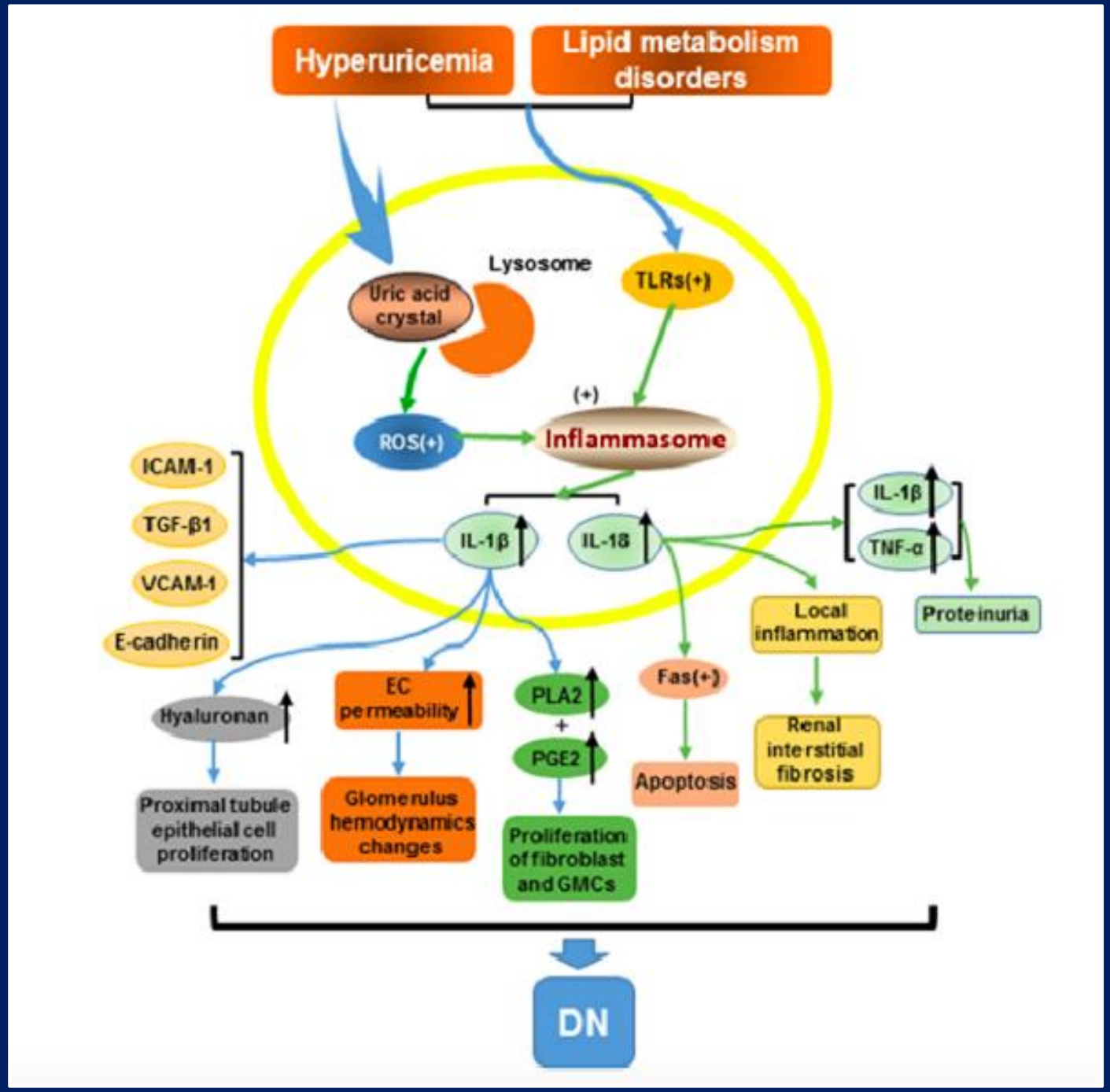
TLRs

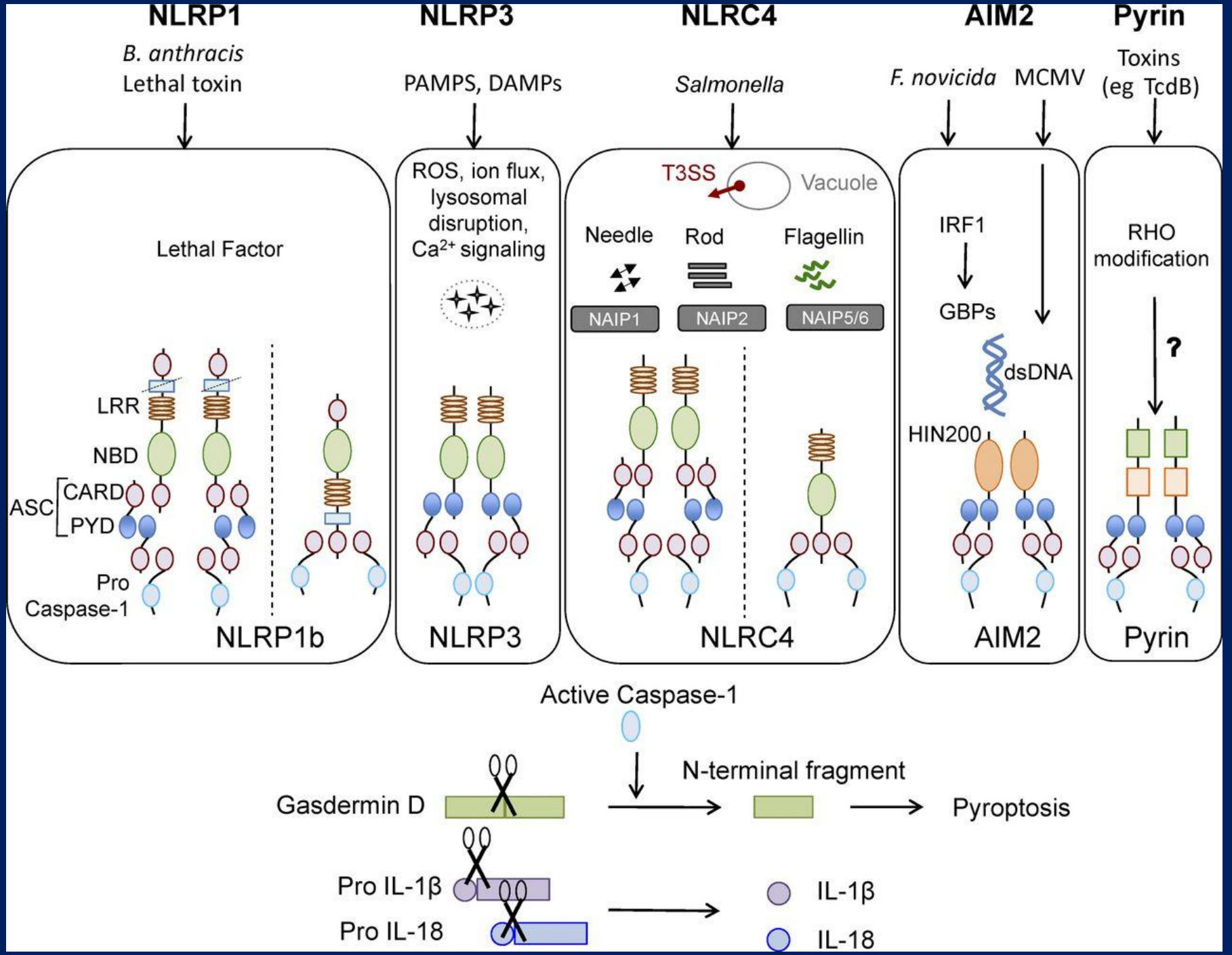

DAMPs
HMGB1, s100/calgranulins, AGEs
Cell Surface
TLR2
TLR4
RAGE
Myddosome complex
MyD88
IRAK1/4
Intracellular Signalling KInases
TAK1
p38 MAPK
JNK
JAK1/2
Transcription Factors
NF-κB
AP-1
ATF-2
STAT1/3
Nucleus
Transcription of Molecules
Promoting Diabetic Nephropathy

Inflammasomes



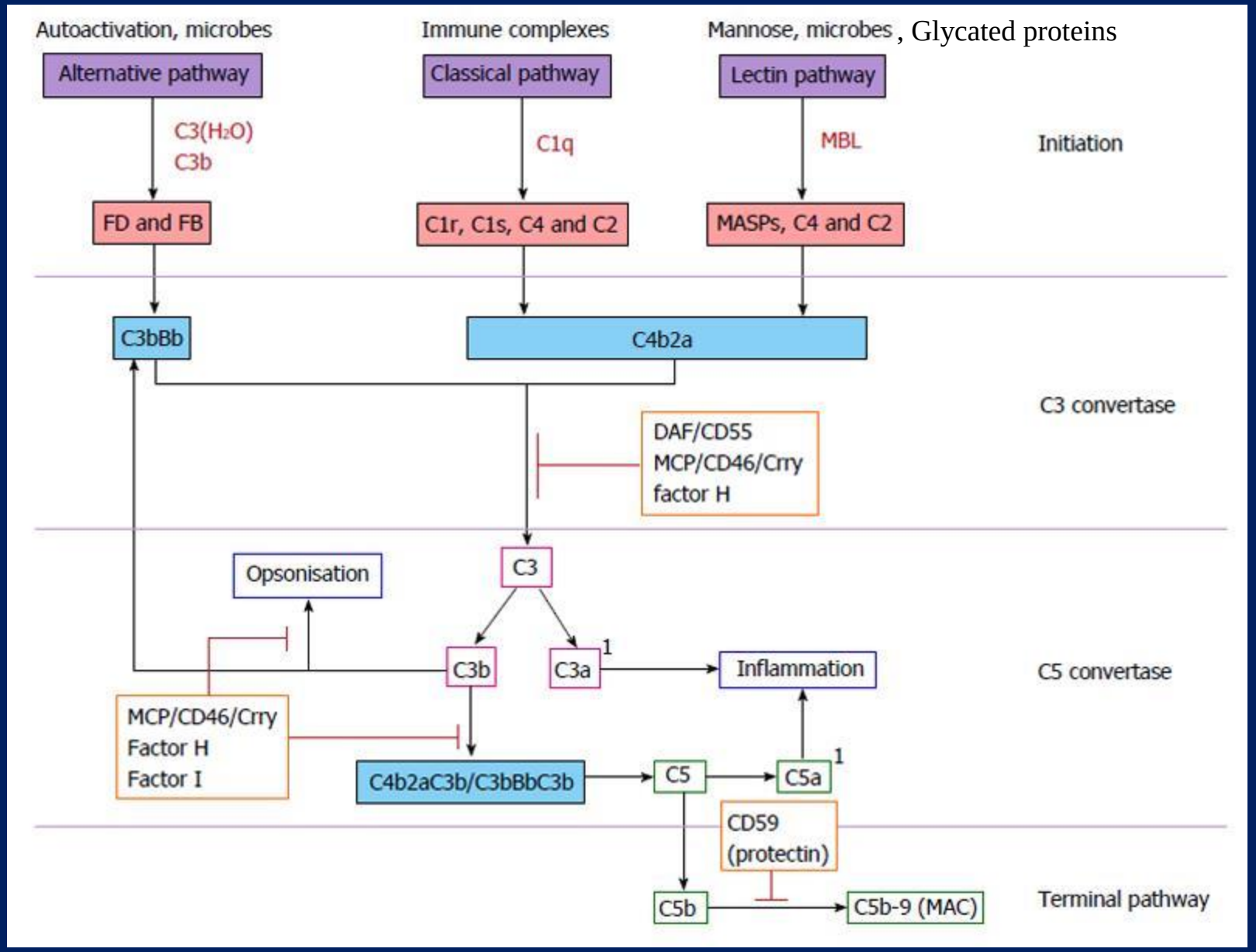

Signal 1:
TLR activation
Signal 2:
Inflammasome
activation by
DAMPS
Pro-IL-1β
Pro-IL-18
NLRP3-ASC
formation
NFκB
dependent
transcription
NUCLEUS
Caspase 1
Active IL-1β
Active IL-18
Saturated FA,
ROS,
Uric acid crystals,
Cholesterol crystals,
Hyperglycemia

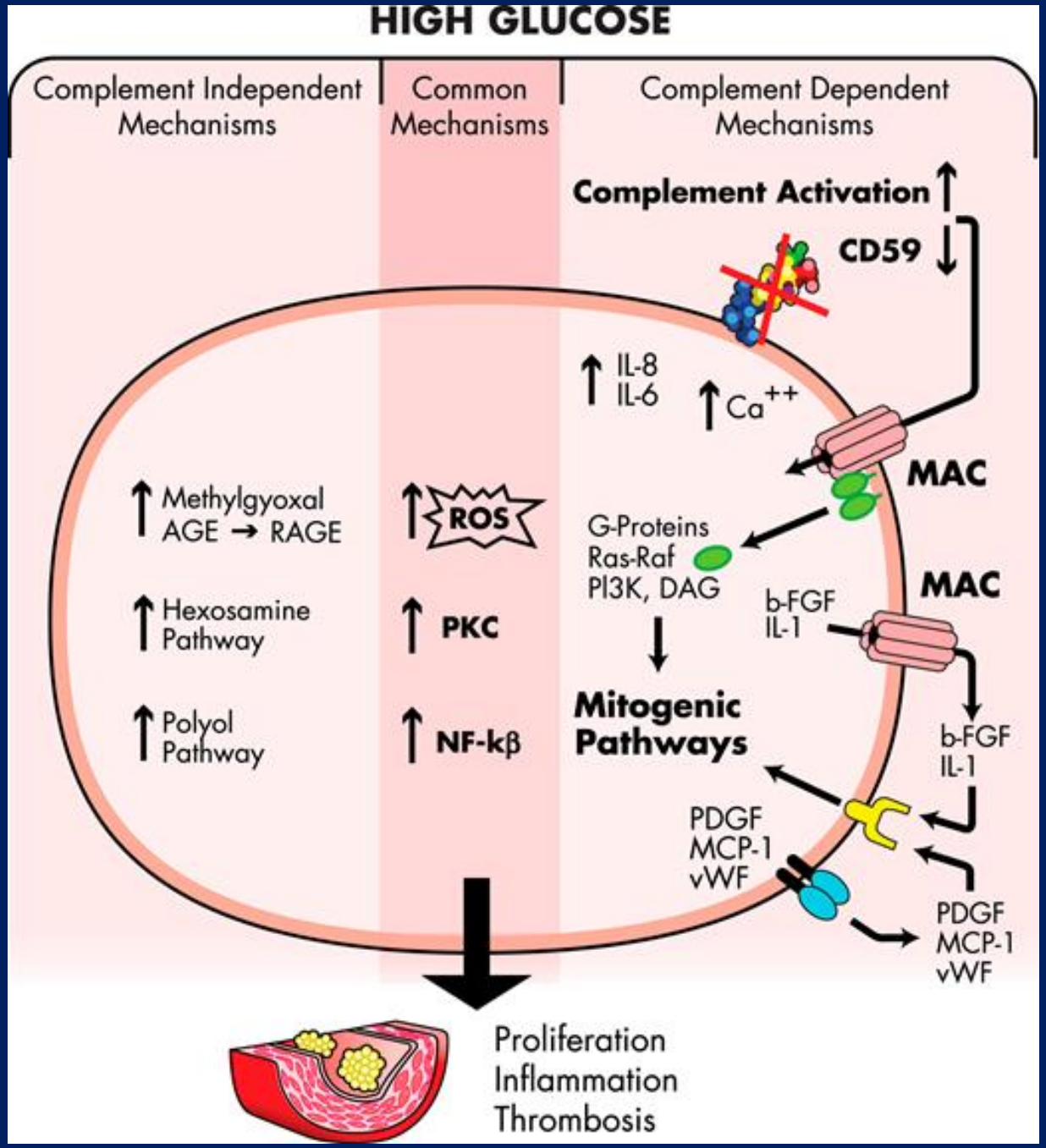

Hyperuricemia
Lipid metabolism disorders
Lysosome
Uric acid crystal
TLRs(+)
(+)
ROS(+)
Inflammasome
ICAM-1
TGF-β1
VCAM-1
E-cadherin
IL-1β
IL-18
IL-1β
TNF-α
Local inflammation
Proteinuria
Hyaluronan
EC permeability
PLA2
+
PGE2
Fas(+)
Apoptosis
Renal interstitial fibrosis
Proximal tubule epithelial cell proliferation
Glomerulus hemodynamics changes
Proliferation of fibroblast and GMCs
DN

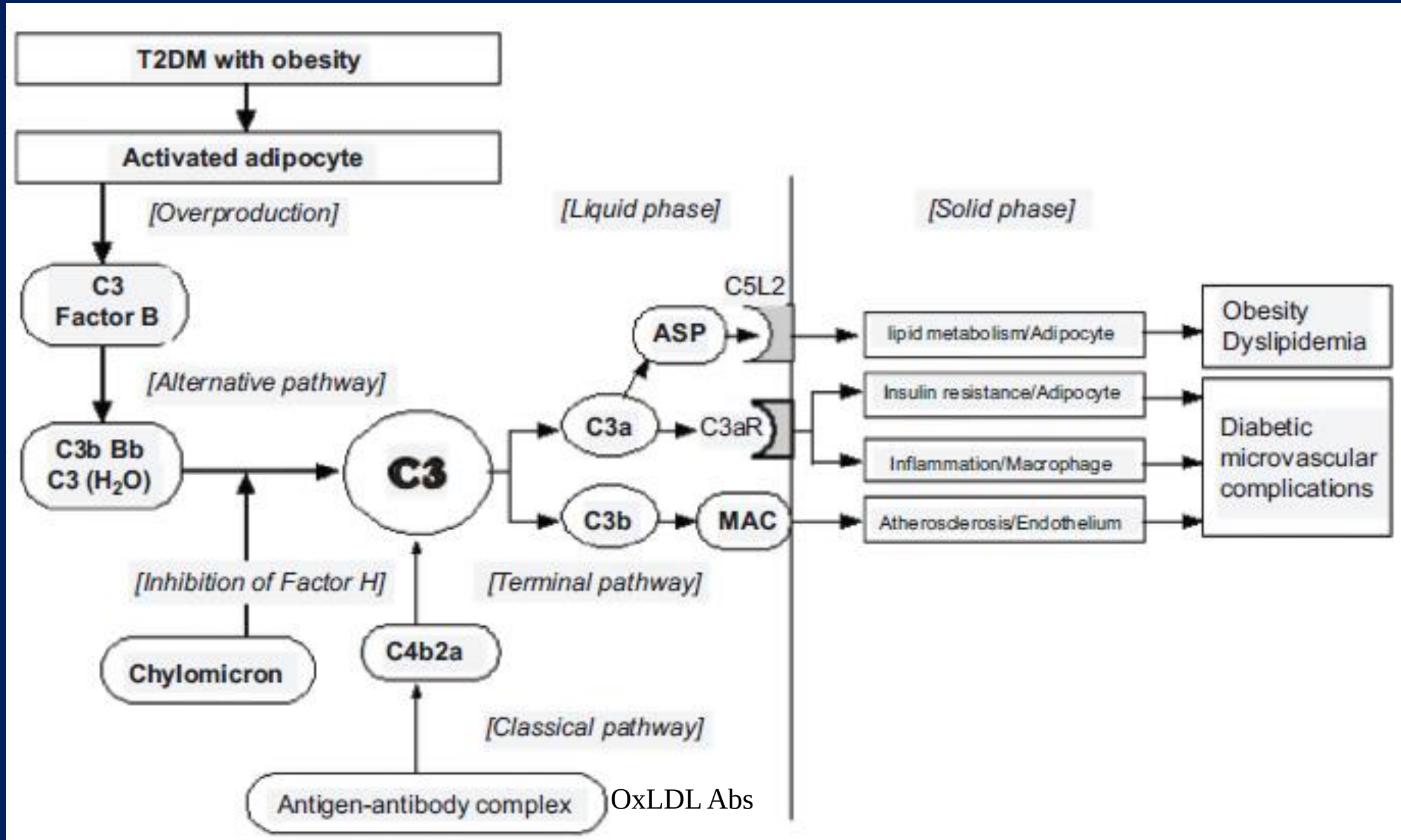

NLRP1
B. anthracis
Lethal toxin
Lethal Factor
LRR
NBD
ASC
CARD
PYD
Pro
Caspase-1
NLRP1b
NLRP3
PAMPS, DAMPs
ROS, ion flux,
lysosomal
disruption,
Ca2+ signaling
NLRP3
NLRC4
Salmonella
T3SS
Vacuole
Needle
Rod
Flagellin
NAIP1
NAIP2
NAIP5/6
NLRC4
AIM2
F. novicida
MCMV
IRF1
GBPs
dsDNA
HIN200
AIM2
Pyrin
Toxins
(eg TcdB)
RHO
modification
?
Pyrin
Active Caspase-1
N-terminal fragment
Gasdermin D
Pyroptosis
Pro IL-1β
Pro IL-18
IL-1β
IL-18

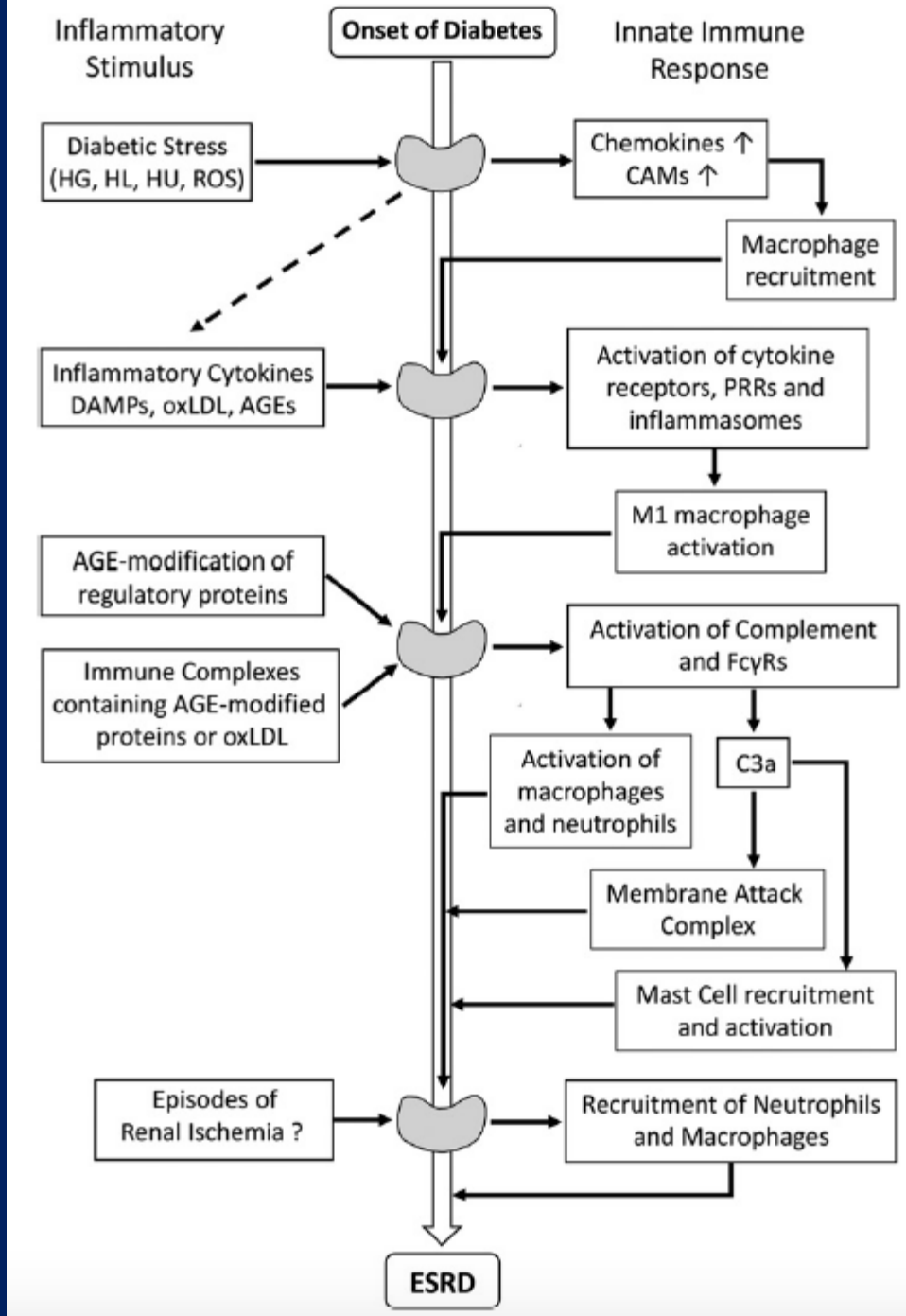
- NLRC4 is a parallel mechanism, in addition to the NLRP3-inflammasome, to induce pro-IL-1β processing and activation.
- The expression of NLRC4 is elevated in DN kidneys.
- NLRC4-deficiency results in diminished DN disease progression, as manifested by a decrease in blood glucose and albumin excretion, as well as preserved renal histology.
- One study demonstrated NLRC4-driven IL-1β production is critical for the progression of DN.

Role of Complement System in Diabetic Nephropathy

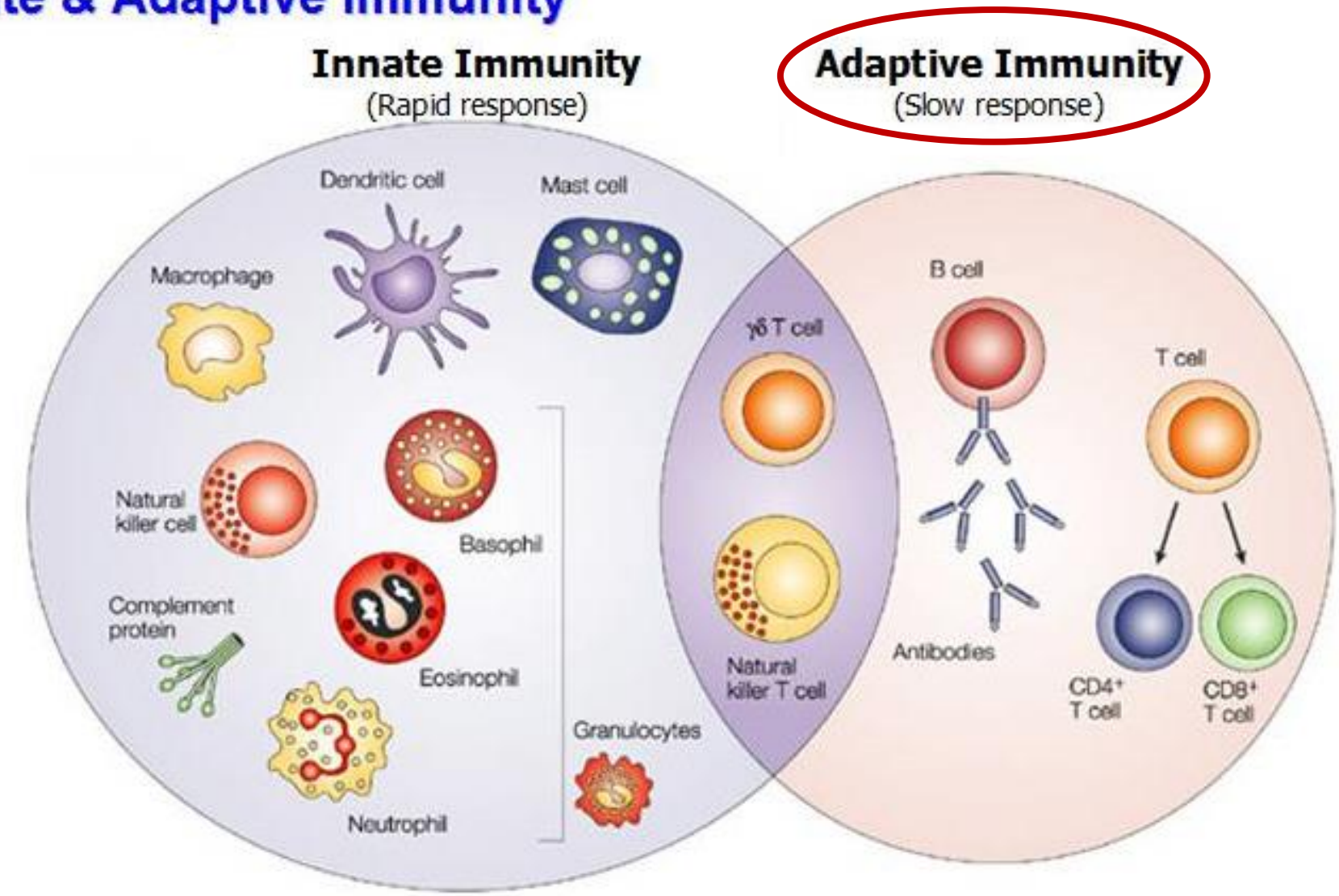

Autoactivation, microbes
Immune complexes
Mannose, microbes , Glycated proteins
Alternative pathway
Classical pathway
Lectin pathway
C3(H2O)
C3b
C1q
MBL
Initiation
FD and FB
C1r, C1s, C4 and C2
MASPs, C4 and C2
C3bBb
C4b2a
C3 convertase
DAF/CD55
MCP/CD46/Crry
factor H
Opsonisation
C3
C3b
C3a
1
Inflammation
C5 convertase
MCP/CD46/Crry
Factor H
Factor I
C4b2aC3b/C3bBbC3b
C5
C5a
1
CD59
(protectin)
C5b
C5b-9 (MAC)
Terminal pathway


HIGH GLUCOSE
Complement Independent Mechanisms
Common Mechanisms
Complement Dependent Mechanisms
Complement Activation
CD59
IL-8
IL-6
Ca++
MAC
Methylgyoxal
AGE → RAGE
ROS
G-Proteins
Ras-Raf
PI3K, DAG
MAC
Hexosamine Pathway
PKC
b-FGF
IL-1
Polyol Pathway
NF-kβ
Mitogenic Pathways
b-FGF
IL-1
PDGF
MCP-1
vWF
PDGF
MCP-1
vWF
Proliferation
Inflammation
Thrombosis


T2DM with obesity
Activated adipocyte
[Overproduction]
C3
Factor B
[Alternative pathway]
C3b Bb
C3 (H2O)
C3
[Inhibition of Factor H]
Chylomicron
C4b2a
[Classical pathway]
Antigen-antibody complex
[Liquid phase]
[Solid phase]
C5L2
ASP
C3a
C3aR
C3b
MAC
[Terminal pathway]
lipid metabolism/Adipocyte
Insulin resistance/Adipocyte
Inflammation/Macrophage
Atherosclerosis/Endothelium
Obesity
Dyslipidemia
Diabetic
microvascular
complications
OxLDL Abs


Inflammatory Stimulus
Onset of Diabetes
Innate Immune Response
Diabetic Stress (HG, HL, HU, ROS)
Chemokines ↑ CAMs ↑
Macrophage recruitment
Inflammatory Cytokines DAMPs, oxLDL, AGEs
Activation of cytokine receptors, PRRs and inflammasomes
M1 macrophage activation
AGE-modification of regulatory proteins
Immune Complexes containing AGE-modified proteins or oxLDL
Activation of Complement and FcγRs
Activation of macrophages and neutrophils
C3a
Membrane Attack Complex
Mast Cell recruitment and activation
Episodes of Renal Ischemia ?
Recruitment of Neutrophils and Macrophages
ESRD

Innate & Adaptive Immunity



Role of T cells in Diabetic Nephropathy

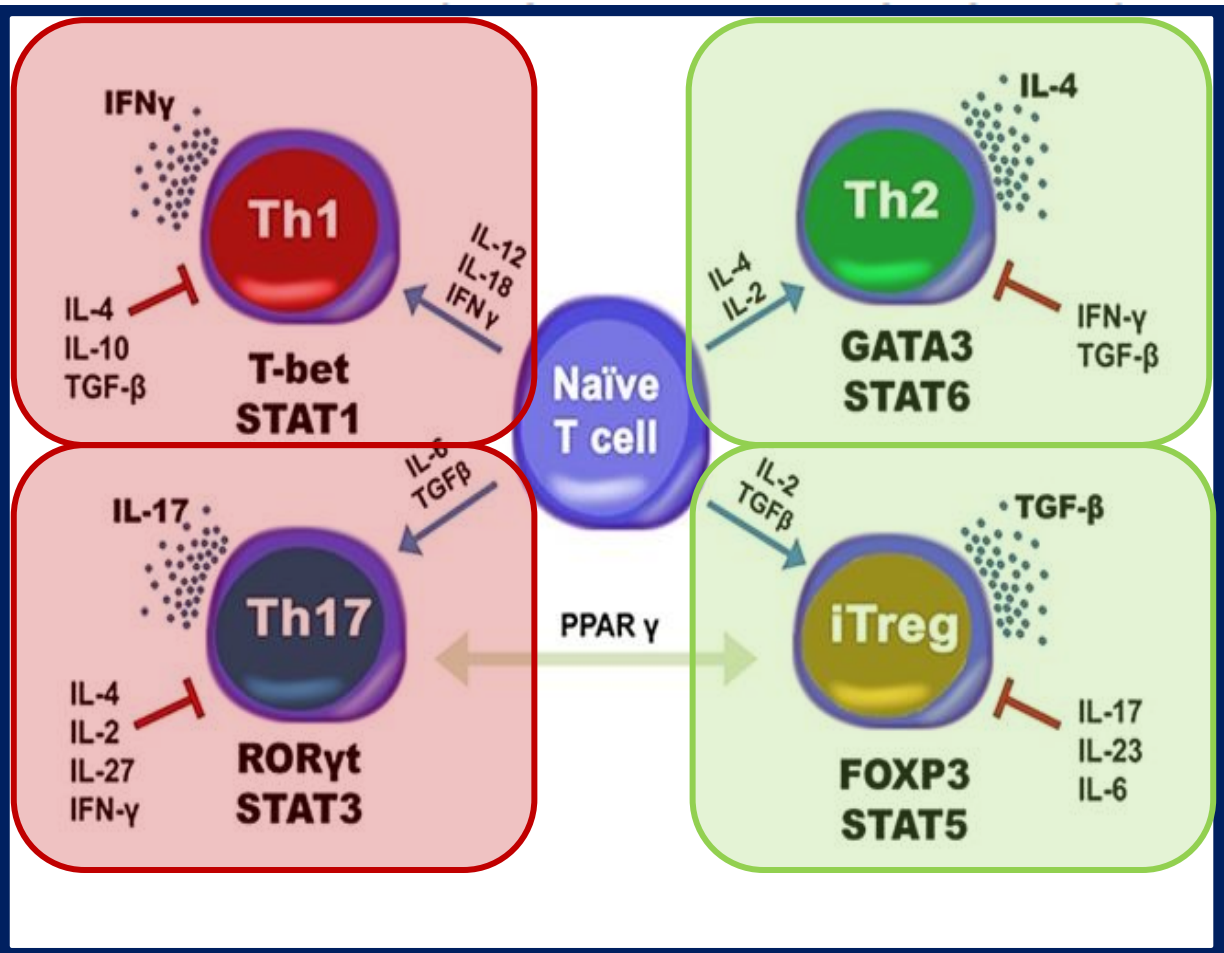
T cells infiltrate the kidneys in DKD

CD4+ and CD8+ T cells and macrophages infiltrate glomeruli and the interstitium in streptozotocin-treated rats (127)

In streptozotocin-treated mice, proliferating CD4+ (74, 131) and CD8+ T cells (131) are increased in the kidney

T-cell accumulate in the juxtaglomerular apparatus in kidneys of people with type 1 diabetes (135, 158). This T cell presence is associated with greater filtration surface per glomerulus (135)

In people with type 2 diabetes, CD4+ and CD8+ T cells accumulate in the interstitium. CD4+ T-cell infiltration is proportional to proteinuria (131)



Tregs improve DKD, while Th1 and Th17 cells worsen DKD

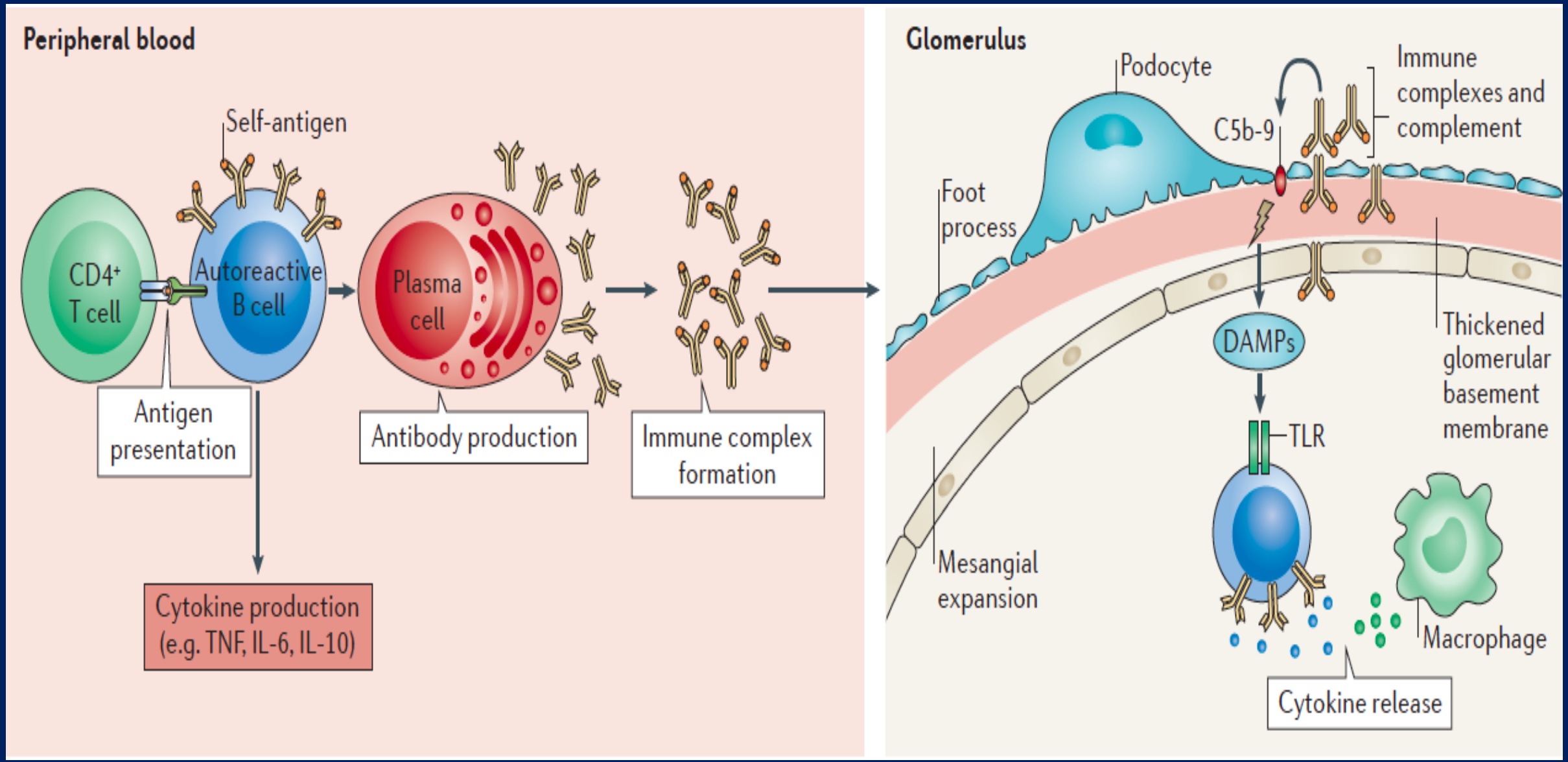
Mice devoid of T and B cells still had matrix expansion, macrophage accrual, tubular injury, and renal function decline but had preserved podocytes and less albuminuria (101)

Mice devoid of T cells (nude mice), treated with streptozotocin, had greater serum TNF-α, kidney hypertrophy, and albuminuria than the wild-type mice (2)

In streptozotocin-treated mice, immunosuppressive treatment with MMF reduced intrarenal IL-17+ CD4+ T cells, albuminuria, and tubulointerstitial fibrosis without affecting glycemia (74)

In *db/db* mice, depletion of Tregs worsened glomerular hyperfiltration and albuminuria, number of CD4+ and CD8+ T cells in the kidney, renal TN-Fα, IFN-γ, and IL-10 mRNA. Adoptive transfer of FoxP3+ Tregs improved albuminuria and glomerular hypertrophy, reduced infiltration of CD8+ T cells, and increased renal FoxP3 and IL-10 expression (58)

Role of B cells in Diabetic Nephropathy


Peripheral blood
Self-antigen
CD4+ T cell
Autoreactive B cell
Plasma cell
Antigen presentation
Antibody production
Immune complex formation
Cytokine production (e.g. TNF, IL-6, IL-10)
Glomerulus
Podocyte
C5b-9
Immune complexes and complement
Foot process
DAMPs
Thickened glomerular basement membrane
TLR
Mesangial expansion
Macrophage
Cytokine release

Novel Avenues for Treating Diabetic Nephropathy

Table 8. *Potential treatment targets*

Treatment Target	Agent	Study Type (Name)	Results
JAK-1, -2	Baricitinib	Phase 2 study	Unpublished to date
CCL2	Spiegelmer NOX-36	Phase 1 and 2 studies	Unpublished to date
CCL2	Bindarit	Phase 2 study	Unpublished to date
CCR2 (CCL2 receptor)	CCX140-B	Randomized, placebo controlled study	Reduction in albuminuria
PKC	Ruboxistaurin	Randomized, placebo controlled, double blind study	Reduction in albuminuria and preservation of GFR
PKC	Antisense oligonucleotides	N/A	N/A
Nrf2	Resveratrol	Phase 2 study	Recruiting patients
AGEs	GLY-230	Phase 2 study	Reduction in albuminuria
AGEs	anti-AGEs DNA aptamer	N/A	N/A
AGEs	Pyridoxamine	Phase 2 study	Unpublished to date
RAGE	Blocking antibodies	N/A	N/A
TNF and other inflammatory mediators	Pentoxifylline	Randomized, placebo controlled, double blind study	Reduction in albuminuria
Serum amyloid A	Antisense oligonucleotides	N/A	N/A
Uric acid	Allopurinol	Randomized, placebo controlled, double blind study	Reduction in albuminuria and preservation of GFR

Drug	Mechanism of action
Pirfenidone	Anti-fibrotic and anti-inflammatory action; reduction of fibroblast proliferation, inhibition of TGF-β-stimulated collagen production, reduction of the production of fibrogenic mediators such as TGF-β and of inflammatory mediators such as TNF-α and IL-1β
Spironolactone	Selective mineralocorticoid (aldosterone) receptor antagonist
Eplerenone	Steroidal antimineralocorticoid of the spirolactone group, similar to the diuretic spironolactone, though it is much more selective for the mineralocorticoid receptor in comparison
Finerenone (BAY94-8862)	Nonsteroidal antimineralocorticoid blocking mineralocorticoid receptors, which makes it a potassium-sparing diuretic
Vitamin D/Paricalcitol	'Activities; VDR activation has been associated with a reduction in IL-6, MCP-1, and TGF-beta levels and ameliorated albuminuria in animal models of DKD. Renal biopsies from T2DM human patients with albuminuria and nondiabetic subjects were compared for VDR expression by immunohistochemistry: VDR expression was downregulated in renal tubular epithelial cells from T2DM patients with albuminuria
N-acetylcysteine	Remarkably active agent shown to be useful in a variety of clinical settings. Its actions include vasodilatation, enhancement of renal medullary blood flow, and antioxidant properties

Thanks for your attention

DKD is associated with an increase in Th1/Th17 and a reduction in Treg activity

In animal models, CD4+ T cells infiltrate the kidneys early in diabetes (1 mo), followed by CD8+ T cells (8 mo). This is associated with higher expression of Th1 cytokines TNF-α and IFN-γ and nitric oxide in the kidney tissue (101, 127)

In streptozotocin-treated mice, there is an increase in IFN-γ and IL-17-producing CD4+ T cells (74)

Streptozotocin-treated mice had an increase in renal CD4+ cells in the kidney interstitium, which produced IFN-γ and TNF-α (131)

People with type 2 diabetes and DKD (29, 211) or microvascular complications (213) had fewer circulating Tregs than controls, with an inverse correlation between Tregs and albuminuria

In people with type 2 diabetes, circulating Th1 and Th17 were increased, proportional to albuminuria. Serum cytokines characteristic of Th1 (IFN-γ, TNF-α, IL-2) and Th17 (IL-17) subsets were also increased in people with DKD, as did IL-10, in correlation with albuminuria. The Th1, Th2, Th17, and Treg proportions were not correlated with A1c (29, 213)

People with type 2 diabetes and DKD had higher circulating Th1 cytokines (IL-2, IL-12, IFN-γ) and lower IL-13 and IL-33, compared with people with diabetes and no DKD (13)

Sevelamer carbonate	Phosphate-binding drug used to treat hyperphosphatemia in patients with chronic kidney disease. When taken with meals, it binds to dietary phosphate and prevents its absorption
Patiromer	Molecule-binding-free potassium ions in the gastrointestinal tract and releasing calcium ions for exchange, thus lowering the amount of potassium available for absorption into the bloodstream and increasing the amount that is excreted via the feces. The net effect is a reduction of potassium levels in the blood serum
Alagebrium	Compound directly targeting the biochemical pathway leading to the stiffness of the cardiovascular system. Removal of the AGEs by cleavage of the abnormal crosslinking bonds has been associated with diminished inflammatory and sclerotic signaling pathway
Resveratrol	Antioxidant effects
Epigallocatechin gallate	Green tea derived molecule with antioxidant effects
Pentoxifylline	Nonselective PDE inhibitor which raises intracellular cAMP, activates PKA, inhibits TNF and leukotriene synthesis, and reduces inflammation and innate immunity
Folic acid	Molecule-reducing oxidative stress and ROS

Role of Neutrophils Diabetic Nephropathy

Variables	Patients with diabetes			
	With early-stage DN (n = 115)	Without early-stage DN (n = 138)	Control group (n = 210)	P-value
WBCs (10^9/l)	6·68 ± 1·95	5·83 ± 1·48	6·46 ± 1·50	<0·001*
Neutrophils (10^9/l)	4·43 ± 1·43	3·72 ± 1·29	3·64 ± 1·20	<0·001*
Lymphocytes (10^9/l)	1·87 ± 0·78	1·79 ± 0·84	2·13 ± 0·60	0·018*
Monocytes (10^9/l)	0·48 ± 0·12	0·47 ± 0·12	0·47 ± 0·11	NS
Platelets (10^9/l)	199·63 ± 47·74	207·18 ± 53·33	207·66 ± 49·02	NS
CRP (mg/l)	2·16 ± 1·10	1·67 ± 0·97	0·97 ± 0·78	<0·001*
NLR	2·48 ± 0·59	2·20 ± 0·62	1·80 ± 0·64	<0·001*

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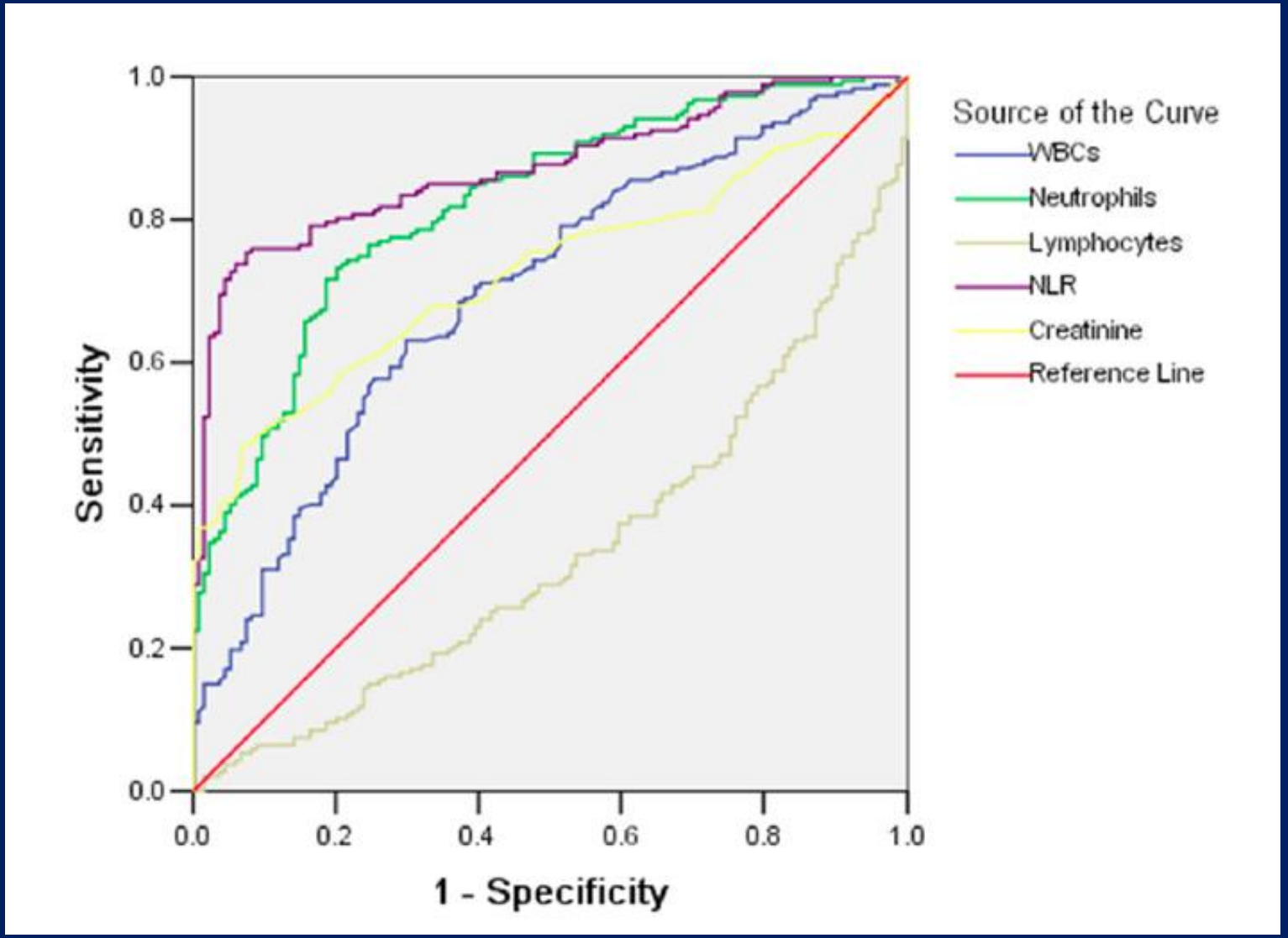

Using a cut-off value of 1.758 for the NLR, the sensitivity was 75.4%, the specificity was 92.5% (the ability of the NLR to predict DN risk).